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Remarks

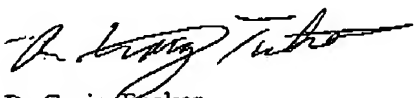
It is first acknowledged with thanks that the Examiner has determined Claims 1, 2, 4, 5, 7, 8, and 11-20 to be allowable.

Claims 9, and 10 currently stand rejected under 35 U.S.C. §112, first paragraph as not being sufficiently enabled commensurate with the scope of those claims. Applicants strongly disagree with the Examiner's characterization. It is known in the art that 5-HT_{2C} receptor activity has been associated with obsessive compulsive disorder as pointed out, among others, in J.R. Martin et al., *5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential*, Journal of Pharmacology & Experimental Therapeutics, 286(2):913-924 (1998)(see particularly pg. 922, last par., and pg. 223, last par.), and M. Bos et al., *Novel agonists of 5HT2C receptors. Synthesis and biological evaluation of substituted 2-(indol-1-yl)-1-methylethylamines and 2-(indeno[1,2-b]pyrrol-1-yl)-1-methylethylamines. Improved therapeutics for obsessive compulsive disorder*, Journal of Medicinal Chemistry, 40(17):2762-9 (1997)(see particularly pg. 2762, 2nd par., point (iii), 4th par, 4th sentence, pg. 2764, 4th and 6th full par.). copies of which are attached hereto for the Examiner's convenience. These reference provides the necessary teaching such that the knowledge that the presently claimed compounds are 5-HT_{2C} receptor agonists together with a modicum of teaching of their formulation and administration, enables their use in the treatment of obsessive/compulsive disorder as claimed in Claims 9 and 10. The combination of the present specification with these references make it obvious how to treat obsessive compulsive disorder by administration of a compound of the present invention. It is not a requirement of enablement under §112 to provide clinical data. Neither is it necessary to specify exact dosages for treatment in that it is quite routine to do the dose ranging studies necessary to determine optimum dosing, which would be expected to vary for any given compound, formulation, and route of administration desired; quantity of experimentation is not the only consideration, but also the nature of the experimention. Here, the necessary dose finding experimentation may require significant resources, but is rudimentary and expected for any given compound/formulation/route of administration. Therefore the rejection of Claims 9 and 10 is improper and its withdrawal is therefore respectfully requested.

Claims 3 and 6 also currently stand rejected under 35 U.S.C. §112, first paragraph. In an effort to facilitate the issuance of the remainder of the Claims, Applicants have cancelled Claims 3 and 6 without prejudice to obviate the rejection, reserving the right to prosecute such claims in a divisional application.

It is believed that all rejections have been overcome or obviated. It is believed that all issues have been addressed and that the Claims are now in condition for allowance. A timely Notice of Allowance is requested.

Respectfully submitted,



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5-HT_{2C} Receptor Agonists: Pharmacological Characteristics and Therapeutic Potential

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ABSTRACT

In vitro, (S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$ and (S)-2-(4,4,7-trimethyl-1,4-dihydro-indeno[1,2-b]pyrrol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$ exhibited high-affinity binding to the serotonin$_{2C}$ (5HT_{2C}) receptors and stimulated turnover of inositol 1,4,5-triphosphate. Affinity to several of the other 5-HT receptor subtypes and to numerous nonserotonergic receptors was much lower. In rats, both compounds elicited behavioral signs of 5-HT_{2C} receptor agonism but not 5-HT_{2A} receptor agonism. Hypomotility induced in rats by high doses of these compounds was reversed by the 5-HT_{2C} receptor antagonist N-(2-naphthyl)-N′-(3-pyridyl)-urea 1:1 HCl. In addition, these compounds were active in tests used to demonstrate anticompulsive effects: reducing schedule-induced polydipsia in rats (prevented by the 5-$HT_{2C/2B}$ receptor antagonist N-(1-methyl-5′-indolyl)-(3-pyridyl)urea 1:1 HCl, reversing increased scratching induced with 8-hydroxy-dipropylaminotetralin 1:1 HCl in squirrel monkeys (no tolerance developed), decreasing responding in the marble-burying task in mice, and decreasing excessive eating of palatable food in rats. In contrast to these compounds, fluoxetine was much less potent, and in some tasks less efficacious, in reducing excessive behavior in these models. These two 5-HT_{2C} receptor agonists do not show anxiogenic effects in the plus-maze in rats. (S)-2-(4,4,7-trimethyl-1,4-dihydro-indeno[1,2-b]pyrrol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$ reduced the olfactory bulbectomy-induced passive avoidance impairment in rats, a result that indicates antidepressant potential. Similarly, in the differential-reinforcement-of-low rate 72-s operant schedule task in rats, (S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$ increased (and (S)-2-(4,4,7-trimethyl-1,4-dihydro-indeno[1,2-b]pyrrol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$ showed a tendency to increase) total reinforcements received, which is suggestive of antidepressant activity. The electroencephalography defined sleep-waking pattern in rats produced by these two 5-HT_{2C} agonists, as well as fluoxetine, included increased quiet-waking and decreased rapid-eye-movement sleep, which is characteristic of antidepressant drugs. These results suggest that 5-HT_{2C} receptor agonism is associated with therapeutic potential in obsessive compulsive disorder and depression.

SSRIs are now well established as efficacious treatment for depression, OCD and bulimia (Den Boer and Westenberg, 1995; Kennedy and Goldbloom, 1994; Rasmussen *et al.*, 1993). Various pharmacological effects of the neurotransmitter 5-HT have been demonstrated to depend predominantly on its interaction with specific 5-HT receptor subtypes (cf. Saxena, 1995). This, in turn, has led to the development of such drugs as buspirone, a 5-HT_{1A} receptor partial agonist, for treatment of anxiety disorders (Goa and Ward, 1986) and ondansetron, a 5-HT_3 receptor antagonist, for reduction of emesis (Milne and Heel, 1991).

5-HT_{2C} receptors are present at very high levels in choroid plexus (as the only 5-HT receptor subtype located there), but they also occur in various other brain regions (Pompeiano *et al.*, 1994). 5-HT_{2C} receptor mRNA is found in many brain areas in addition to those autoradiographically shown to have binding sites. 5-HT_{2C} mRNA was located in noradrenergic, dopaminergic and cholinergic nuclei. Lower, but nonetheless still high, receptor densities are found in the limbic system and cortex (especially frontal cortex), a result consistent with a possible important role of 5-HT_{2C} receptors in

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ABBREVIATIONS: m-CPP dihydrochloride, 1-(3-chlorophenyl)piperazine 1:2 HCl; 5-CT, 5-carboxamidotryptamine; DOB, 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane; (±)-DOI hydrochloride, rac-4-iodo-2,5-dimethoxy-α-methylbenzylamine 1:1 HCl; 8-OH-DPAT hydrochloride, 8-hydroxy-dipropylaminotetralin 1:1 HCl; SB200646A, N-(1-methyl-5′-indolyl)-(3-pyridyl)urea 1:1 HCl; Ro 60-0175/ORG 35030, (S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$; Ro 60-0332/ORG 35035, (S)-2-(4,4,7-trimethyl-1,4-dihydro-indeno[1,2-b]pyrrol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$; Ro 60-0491/001, N-(2-naphthyl)-N′-(3-pyridyl)-urea 1:1 HCl; 5-HT, serotonin; DRL-72 s, differential-reinforcement-of-low-rate 72-s operant schedule; EEG, electroencephalogram; EMG, electromyogram; FT-1 min, fixed-time 1-min operant schedule; IP_3, inositol 1,4,5-triphosphate; OCD, obsessive compulsive disorder; p.o., per os; REM, rapid eye movement; SSRI, selective serotonin reuptake inhibitor.

affective disorders. Specific antibodies have also been used to study the location of 5-HT_{2C} receptors, recognizing sites in rat choroid plexus, hippocampus, cerebral cortex, striatum and substantia nigra and in human cerebral cortex, substantia nigra and cerebellum (Abramowski *et al.*, 1995).

The potential use of 5-HT_{2C} receptor ligands in psychiatry is suggested by a number of observations. Transgenic mice that lacked functional 5-HT_{2C} receptors exhibited abnormal feeding behavior leading to overweight, as well as susceptibility to seizures (Tecott *et al.*, 1995). 5-HT_{2C} receptor-mediated effects, such as hypophagia, resemble those induced by SSRIs (Leander, 1987; Lucki *et al.*, 1988), and furthermore, the interoceptive cue produced by SSRIs has been reported to exhibit similarity to that of the 5-HT_{2C} receptor agonist MK212, only partially to the 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT and not to the 5-HT_{2A} receptor agonist DOI (Berendsen and Broekkamp, 1994). Such results suggest that some of the therapeutic effects of the SSRIs may be mediated at least in part by 5-HT_{2C} receptor agonism. Certainly there are a number of common effects in rodents that have now been noted for SSRIs and 5-HT_{2C} receptor agonists, including inhibition of escape from aversive periaqueductal gray stimulation, hypophagia, decreased defensive burying, induction of penile erection and inhibition of muricide (Broekkamp and Berendsen, 1992). A considerable body of preclinical and clinical data on m-CPP, which exhibit high-affinity binding to 5-HT_{2C} receptors (as well as affinity to several other 5-HT receptor subtypes and nonserotonergic receptors) indicates that under some circumstances it exacerbates clinical symptoms in patients with OCD or panic anxiety (Kahn and Wetzler, 1991). However, recent results suggest that such effects are probably 5-HT_{1D} receptor-mediated (Zohar and Cohen, 1995; Loi *et al.*, 1995). Unfortunately, only nonselective 5-HT_{2C} receptor agonists have been available as research tools. The present study provides an extensive biochemical and pharmacological characterization of two novel 5-HT_{2C} receptor agonists.

Novel agents acting at the central 5-HT_{2C} receptors may offer therapeutic advantages. The absence of appreciable affinity to certain other 5-HT subtypes may provide the basis for an improved therapeutic index compared with the general activation of all the different 5-HT receptor subtypes by SSRIs. For example, 5-HT_{1A} receptor agonism has been associated with evidence of sexual dysfunction and anxiogenesis in animals (*e.g.*, Ahlenius *et al.*, 1986; Moser *et al.*, 1990; Maswood *et al.*, 1996), and 5-HT_{2A} receptor agonism has been implicated in cardiovascular effects (Saxena, 1995). The experimental results described here with novel selective 5-HT_{2C} receptor agonists in different animal models of psychiatric disorders support this view. Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 are 5-HT_{2C} receptor agonists that offer the potential advantages of therapeutically relevant effects in animal models of OCD and depression similar to or better than those of fluoxetine, combined with an improved side-effect profile. SSRIs enhance endogenously released 5-HT and thereby activate all postsynaptic 5-HT receptor subtypes. A direct agonistic effect at the 5-HT_{2C} receptor (especially in the absence of any appreciable 5-HT_{2A} receptor agonism) defines the probable mechanism responsible for the favorable pharmacological profiles of Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035.

Materials and Methods

In Vitro Pharmacology

***In vitro* binding assays.** In most binding assays, the compounds were tested at a single concentration (10^{-6} M) using the methodology described in table 1. In those assays where the compounds displaced more than 50% of the specific binding, the compounds were retested at multiple concentrations to estimate pK_i values. With respect to the 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors, experiments were performed with membranes obtained from NIH 3T3 cell lines expressing human 5-HT_{1A}, human 5-HT_{2A} or human 5-HT_{2C} receptors (kindly donated by Dr. Nico Stam, N.V. Organon). For each receptor subtype, a single batch of cells was grown using fermentation techniques previously described (Sleight *et al.*, 1996). Radioligand binding assays were as previously described for the human 5-HT_{2A} receptor, with minor modifications for labeling human 5-HT_{1A} and human 5-HT_{2C} receptors. Briefly, on the day of the experiment, membranes were thawed and resuspended in 10 times the original volume of assay buffer to give a concentration of approximately 4×10^5 cells per assay tube. The assay buffer consisted of Tris-HCl 50 mM, pargyline 10^{-5} M, $MgCl_2$ 5 mM and ascorbic acid 0.1%, pH 7.4. All compounds were dissolved in 10% dimethyl sulfoxide and diluted in assay buffer. Assays were similar for each receptor and consisted of 100 μl of membrane preparation (depending on the assay), 50 μl of radioligand ([^{3}H]-5-HT 1 nM final concentration for labeling human 5-HT_{1A} and human 5-HT_{2C} receptor binding sites and [^{3}H]-DOB 1 nM final concentration for labeling human 5-HT_{2A} receptors). Nonspecific binding was defined in the presence of 10 μM 5-HT in the case of the human 5-HT_{1A} and 5-HT_{2C} receptor and in the presence of 10 μM methysergide in the case of the human 5-HT_{2A} receptor. The specific activities of [^{3}H]-5-HT and [^{3}H]-DOB were 29.7 and 15.0 Ci/mmole, respectively (New England Nuclear, Boston, MA). The incubations were performed at room temperature for 1 h. For all binding experiments, reactions were stopped by rapid filtration through either Whatmann GF/B or GF/C filters. Filters were washed with 3 × 2 ml of Tris-HCl (50 mM, pH 7.4), and the radioactivity retained on the filters was measured by scintillation spectroscopy. All displacement experiments were performed in triplicate and were repeated at least three times. Saturation analyses were performed for each receptor using at least eight concentrations of each radioligand (concentrations ranging from 0.05 to 10 nM). Dissociation constants (K_d) were calculated using the EBDA/LIGAND program (McPherson, 1985; Munson and Rodbard, 1980). Where displacement experiments were performed, curves were constructed using seven concentrations of the displacing agents (one data point per logarithmic unit of concentration: 10^{-11} to 10^{-5} M). Displacement curves were analyzed using EBDA/LIGAND to calculate pK_i values.

Tissue preparation and incubation for measurement of IP_3 production. 5-HT_{2C}-mediated stimulation of IP_3 production was measured in the choroid plexus of the rat. The choroid plexus was removed, placed in 200 μl of oxygenated Krebs solution and incubated with 0.35 nmol myoinositol and 0.35 nmol [^{3}H]-myoinositol for 1 h at 37°C. During this incubation, the tubes were gassed with 95% oxygen/5% CO_2 every 20 min. A mixture of LiCl and pargyline was then added (final concentration: LiCl = 10 mM, pargyline = 10 μM), followed 10 min later by addition of the test compounds (final incubation volume = 250 μl). Dose-response curves were constructed from data obtained from three separate measurements per data point. The mixture was incubated for a further 30 min at 37°C. The assays were stopped by the addition of 25 μl of a stopping solution ($HClO_4$ 2.64 N + EDTA 40 mM). Assay tubes were frozen on dry ice for 15 min, thawed and then kept on ice for 1 h. The tubes were then centrifuged for 20 min at 24,000 × g. Then 250 μl of the supernatant was removed and placed in Eppendorf tubes together with 225 μl 4 M KOH. The samples were mixed well and kept on ice for 15 min. These samples were then recentrifuged for 15 min at 14,000 rpm, 220 μl of supernatant was removed and 30 μl of phytic acid was added. The

TABLE 1
Assay conditions for radioligand binding experiments

Receptor	Ligand (concentration)	Tissue	Incubation Conditions	Nonspecific Ligand (concentration)
5-HT1A	[^{3}H]5-HT (1 nM)	human recombinant (3T3 cells)	1 h/25°C	serotonin (10 μM)
5-HT1B	[^{125}I]CYP (0.1 nM) + isoprenaline (30 μM)	rat cerebral cortex	1.5 h/37°C	serotonin (10 μM)
5-HT1D	[^{3}H]LSD (2 nM)	human recombinant (HEK 293 cells)	1 h/25°C	serotonin (10 μM)
5-HT2A	[^{3}H]DOB (1 nM)	human recombinant (3T3 cells)	1 h/25°C	methysergide (10 μM)
5-HT2C	[^{3}H]5-HT (1 nM)	human recombinant (3T3 cells)	1 h/25°C	serotonin (10 μM)
5-HT3	[^{3}H]BRL 43694 (1 nM)	N1E-115 cells	30 min/25°C	metoclopramide (100 μM)
5-HT4	[^{3}H]GR 113808 (0.1 nM)	guinea pig striatum	1 h/37°C	serotonin (30 μM)
5-HT6	[^{3}H]LSD (1 nM)	human recombinant (Hela cells)	1 h/37°C	serotonin (10 μM)
5-HT7	[^{3}H]LSD (2 nM)	human recombinant (CHO cells)	1 h/37°C	Methiothepin (10 μM)
Adenosine A1	[^{3}H]CCPA (0.5 nM)	rat cerebral cortex	2 h/25°C	CPA (10 μM)
Adenosine A2	[^{3}H]CGS-21680 (6 nM)	rat striatum	1.5 h/27°C	NECA (100 μM)
α-1 adrenergic	[^{3}H]prazosin (0.25 nM)	rat cerebral cortex	1 h/25°C	prazosin (0.5 μM)
α-2 adrenergic	[^{3}H]RX821002 (0.5 nM)	rat cerebral cortex	30 min/25°C	(−)epinephrine (100 μM)
β-1 adrenergic	[^{3}H](−)CPG 12177 (1 nM)	rat heart	20 min/25°C	alprenolol (50 μM)
β-2 adrenergic	[^{3}H](−)CPG 12177 (1 nM)	guinea pig lung	20 min/25°C	alprenolol (50 μM)
	[^{3}H](−)CPG 12177 (0.15 nM)	human recombinant (SF9 cells)	20 min/25°C	alprenolol (50 μM)
Dopamine D-1	[^{3}H]SCH 23390 (0.5 nM)	human recombinant (GH4 cells)	45 min/25°C	butaclamol (10 μM)
Dopamine D-2 [long form]	[^{3}H]spiperone (0.2 nM)	human recombinant (CHO cells)	1 h/25°C	butaclamol (10 μM)
Dopamine D-3	[^{3}H]YM-09151-2 (0.1 nM)	rat recombinant (CHO cells)	1 h/27°C	butaclamol (10 μM)
Dopamine D-4 [D4.4R isoform]	[^{3}H]spiperone (0.2 nM)	rat recombinant (CHO cells)	1 h/25°C	butaclamol (10 μM)
Dopamine D-5	[^{3}H]SCH 23390 (1 nM)	rat recombinant (CHO cells)	1 h/25°C	SCH 23390 (10 μM)
Histamine H-1	[^{3}H]pyrilamine (0.5 nM)	guinea pig cerebellum	10 min/25°C	triprolidine (100 μM)
Histamine H-2	[^{3}H]tiotidine (2 nM)	guinea pig cerebral cortex	30 min/25°C	histamine (5 μM)
Histamine H-3	[^{3}H](R)α-methylhistamine (0.5 nM)	rat cerebral cortex	1 h/25°C	(R)α-methylhistamine (5 μM)
Muscarinic M-1	[^{3}H]pirenzepine (0.6 nM)	rat cerebral cortex	2 h/25°C	atropine (1 μM)
	[^{3}H]pirenzepine (2 nM)	human recombinant (CHO cells)	1 h/25°C	atropine (1 μM)
Muscarinic M-2	[^{3}H]AF-DX 384 (2 nM)	rat heart	1 h/25°C	atropine (1 μM)
	[^{3}H]AF-DX 384 (3 nM)	human recombinant (CHO cells)	1 h/27°C	atropine (1 μM)
Muscarinic M-3	[^{3}H]4-DAMP (0.2 nM)	rat salivary gland	45 min/25°C	atropine (1 μM)
	[^{3}H]4-DAMP (0.1 nM)	human recombinant (CHO cells)	1 h/27°C	atropine (1 μM)
Muscarinic M-4	[^{3}H]4-DAMP (0.2 nM)	human recombinant (CHO cells)	1 h/27°C	atropine (1 μM)
Muscarinic M-5	[^{3}H]4-DAMP (0.5 nM)	human recombinant (CHO cells)	1 h/25°C	atropine (1 μM)
Nicotinic	[^{3}H]cytisine (1 nM)	rat cerebral cortex	75 min/4°C	nicotine (10 μM)
Kainate	[^{3}H]kainate (5 nM)	rat cerebral cortex	1 h/4°C	L-glutamate (1 μM)
AMPA	[^{3}H]AMPA (5 nM)	rat cerebral cortex	1 h/4°C	L-glutamate (1 μM)
Mu opiate	[^{3}H]DAMGO (1 nM)	rat cerebral cortex	1 h/25°C	naloxone (1 μM)
Delta opiate	[^{3}H]PCl-Phe-DPDPE (0.75 nM)	rat cerebral cortex	15 h/25°C	naltrexone (10 μM)
Kappa opiate	[^{3}H]U-69593 (1.5 nM)	guinea pig cerebellum	1 h/25°C	naloxone (10 μM)
BZD	[^{3}H]flumazenil (1 nM)	rat cerebral cortex	1 h/4°C	diazepam (10 μM)

isolation of IP_3 was as described in a previous report (Bourson *et al.*, 1996). A concentration-response curve was constructed for 5-HT, m-CPP and the test compounds. Six concentrations were used per test compound, the highest concentration tested being 0.1 mM. The maximal effect produced by each compound was compared with the stimulation induced by 10 μM 5-HT to calculate the relative intrinsic activity.

In Vivo Pharmacology

Animals and maintenance conditions. Adult male and female MORO mice and 3-week-old female DBA/2J mice (Biological Research Laboratories, CH-4414 Füllinsdorf, Switzerland) and male Swiss mice (Charles River, Sulzfeld, Germany) were used. Adult male and female RORO rats (Biological Research Laboratories, CH-4414 Füllinsdorf, Switzerland), male and female Sprague-Dawley rats (Biological Research Laboratories, CH-4414 Füllinsdorf, Switzerland and Charles River, Sulzfeld, Germany) and male Long-Evans rats (Hsd/Cpbs: Harlan; Zeist, The Netherlands) were used. The mice and rats were delivered to the laboratory colony at least one day before testing. These animals were housed in group cages with sawdust bedding under standard maintenance conditions (12:12 h light-dark cycle; 21–23°C; 55–65% relative humidity). Both mice and rats received laboratory chow and tap water *ad libitum* in the home cage (except when otherwise specified by the experimental procedure). All testing was done during the light portion of the day-night cycle. At the conclusion of testing, the rodents were euthanized by CO_2 exposure.

Adult male squirrel monkeys (*Saimiri sciureus*) of approximately 1 kg b.w.t. were used. The monkeys were maintained in an isolated facility under the supervision of veterinary staff. All monkeys had been in the laboratory colony for several years and were drug-experienced. Before the start of any of the present experiments, the monkeys had received no drug treatment for at least 1 month. The monkeys were maintained either in groups of 6 to 12 in a room-size volière or in pairs in stainless steel cages (0.85 m × 0.6 m × 1.2 m) with two elevated platforms and a hanging chain for climbing. Both the temperature (28–30°C) and the humidity (50–65%) of the animal quarters were regulated. A 12:12 hour light-dark cycle with light onset at 6 A.M. was used. A dry, nutritionally sufficient diet was available *ad libitum* and was supplemented each day with fresh fruits and vegetables. Tap water was available continuously in the home cage.

Behavioral observation in rats. Adult male RORO rats were injected s.c. with vehicle, Ro 60-0175/ORG 35030 or Ro 60-0332/ORG 35035, as well as the reference compounds (±)-DOI and m-CPP and then placed individually in Macrolon cages (29 × 28 × 9 cm) with sawdust bedding for observation of the intrinsic effects of each test compound. Groups of up to eight rats representing different treatment conditions were observed simultaneously. A mirror placed behind the cages permitted an all-round view of the rats. The experimental compounds were given in 0.3% (w/v) Tween 80 in distilled water in a volume of 2 ml/kg b.wt. The doses 0.1, 0.22, 1, 3.2 and 10 mg/kg were evaluated (m-CPP was also tested at the doses 0.01 and 0.032 mg/kg). The rats were drug-experienced (at least 3 days wash-

out between successive tests). Observation began 5 min after injection. The presence or absence of penile erection (see Berendsen and Gower, 1986) was scored during successive 5-min intervals for 40 min. Head shakes (total number of separate bouts) were scored during four 5-min blocks at 5, 15, 25 and 35 min after injection and cumulated for the whole session. These behavioral signs provide a preliminary *in vivo* indication of 5-HT_{2C} receptor (*i.e.*, penile erection) and 5-HT_{2A} receptor (*i.e.*, head shake) activation (Berendsen *et al.*, 1990). The ED_{50} value was calculated by probit analysis.

Behavioral observation in squirrel monkeys. Adult male squirrel monkeys were administered p.o. different doses of fluoxetine (1, 3, 10 or 30 mg/kg), Ro 60-0175/ORG 35030 (0.3, 0.6, 1, 3 or 10 mg/kg), Ro 60-0332/ORG 35035 (0.3, 1, 3 or 10 mg/kg), or vehicle and then placed in an individual stainless steel cage (0.85 m × 0.6 m × 1.2 m) with two elevated platforms and a hanging chain for climbing. Groups of 5 to 10 monkeys per treatment condition were used. The test compounds were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 2 ml/kg b.wt. Behavioral observations of the squirrel monkeys by a trained observer began immediately after administration and continued over a period of at least 6 h.

Spontaneous motor activity in rats. Locomotor activity was monitored in naive adult male RORO rats *via* a Digiscan Animal Activity Monitoring System (Omnitech Electronics, Columbus, Ohio). The test compounds were administered in 0.3% (w/v) Tween-80 in distilled water in a volume of 2 ml/kg b.wt. All testing was carried out in an illuminated room during the light portion of the day-night cycle. The parameter total distance (centimeters) was measured. The 5-HT_{2C} receptor antagonist Ro 60-0491 (pK_i values: h5-HT_{2C} = 7.7; h5-HT_{2A} = 6.0, reversal of m-CPP induced penile erection: ID_{50} = 7.7 mg/kg s.c.; unpublished results) at the dose 10 mg/kg or vehicle was injected i.p., followed 15 min later by a second i.p. injection of vehicle, m-CPP (6 mg/kg), Ro 60-0175/ORG 35030 (10 mg/kg) or Ro 60-0332/ORG 35035 (10 mg/kg). Immediately after the second injection, each rat was placed into an individual Plexiglas test cage (41 × 41 × 28 cm) with sawdust bedding, and its activity was measured for a 30-min period. A different group of eight rats was used for each treatment condition. Statistical comparison was done with a two-tailed Mann-Whitney *U* test with a P value ≤ .05 accepted as significant.

Anticonvulsant effects in mice. Protection from audiogenic seizures was investigated in naive female 21-day-old DBA/2J mice (a strain that exhibits age-dependent susceptibility to seizure). Oral treatment with a test compound or vehicle was given 30 min before testing. Ro 60-0175/ORG 35030 (21, 32, 46, 68, 100 or 150 mg/kg) and Ro 60-0332/ORG 35035 (3.2, 10, 32 or 100 mg/kg) were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 10 ml/kg b.wt. After treatment, each mouse was placed in a separate open transparent Plexiglas box composed of half of a circular container (diameter = 40 cm, height = 20 cm) containing sawdust bedding. Testing was done in a sound-isolated chamber and involved exposure to a 14-kHz sinusoidal tone at 95 dB measured 2 cm above the floor (zero dB was defined as a pressure level of 20 μPa) for 1 min, during which observations were made. After vehicle treatment, such acoustic stimulation typically induced wild running, clonic seizures and tonic convulsions in approximately 90% of the mice tested. Groups of 6 to 8 mice were used to evaluate each dosage condition and the vehicle condition, and the proportion of the group that failed to exhibit tonic convulsions was recorded. ED_{50} values were calculated by probit analysis.

Elevated plus-maze task in rats. Exploration was measured in an elevated plus-maze task in adult naive Sprague-Dawley male rats weighing 110 to 200 g at the time of testing. Drugs that increase exploration within this situation (*i.e.*, benzodiazepine anxiolytics) are considered to exhibit anxiolytic-like effects. The apparatus was 50 cm above the floor and consisted of two open arms (50 cm × 10 cm) perpendicular to two closed arms (50 cm × 10 cm × 50 cm high) extending from an open central area (10 cm × 10 cm). The light intensity on the central platform was 225 lux. All parts of the apparatus were made of grey polyvinylchloride plastic. The effects of i.p. administration of fluoxetine (5 mg/kg), Ro 60-0175/ORG 35030 (1.5 mg/kg) and Ro 60-0332/ORG 35035 (7.5 mg/kg) were evaluated. The test compounds were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 5 ml/kg b.wt. Thirty minutes after injection, the rat was placed in the center of the plus-maze facing one of the closed arms and observed for 5 min *via* a closed-circuit television camera by an observer located in an adjacent room. Number of entries and total time spent in the open arms were measured. The number of closed-arm entries was also recorded as a parameter of general locomotion, and the number of attempts at entry into open arms followed by avoidance behavior was recorded as a measure of risk assessment. Entry into an arm was defined as the rat placing all four paws into the arm. The floor of the maze was thoroughly cleaned after each test trial. The effects of both the first injection and the final injection in a series of four successive daily injections were investigated. During the subchronic experiment, the rats were group-housed under the previously described maintenance conditions with five rats per cage. Each treatment group was composed of 16 rats. Differences between vehicle and drug treatments were evaluated with a single-factor ANOVA followed by a Bonferroni/Dunn *post-hoc* test for multiple comparisons with a P value ≤ .05 accepted as statistically significant.

Burying behavior in mice. In this assessment, the mice are individually placed in a cage with glass marbles located on a layer of sawdust bedding; the marbles make it easy to quantify burying behavior. Naive adult Swiss mice were tested in a Macrolon-type II test cage (24 × 18 × 13 cm) containing 25 marbles (1.5 cm in diameter) placed together in the middle on a 5-cm layer of sawdust. Fluoxetine (10, 22 or 46 mg/kg), Ro 60-0175/ORG 35030 (2.2, 4.6 or 10 mg/kg) and Ro 60-0332/ORG 35035 (4.6, 10 or 22 mg/kg) were evaluated. Vehicle or different doses of each experimental compound were administered s.c. 30 min before testing. The experimental compounds were given in 5% Mulgofen (EL 719R, GAF Corp., New York, NY) in physiological saline. The volume of administration was 10 ml/kg b.wt. Groups of nine mice were used to evaluate each treatment condition. The mice were placed in individual test cages for a 30-min test. The number of marbles covered at least two-thirds by sawdust was counted at the conclusion of testing. The median for each dosage group was calculated. The ED_{50} values were calculated by probit analysis.

Palatable food intake in rats. Adult female RORO rats were used to evaluate hypophagia in a palatable food paradigm. The nondeprived rats were repeatedly given boiled potato in separate Macrolon test cages (30 × 25 × 10 cm) lacking bedding until a stable level of consumption was obtained during the 30-min test on several successive days. Subsequently, the same group of drug-experienced rats were tested with vehicle and with all dosage conditions for a given test compound, a minimum of 2 days intervening between successive tests. The compounds tested were fluoxetine (3, 10, 30 or 60 mg/kg), m-CPP (0.1, 0.3, 1 or 3 mg/kg), Ro 60-0175/ORG 35030 (0.3, 1, 3 or 10 mg/kg) and Ro 60-0332/ORG 35035 (10, 30 or 60 mg/kg). Several test sessions done with vehicle were interspersed with those of the different doses of a test compound (a mean value was then calculated). These test compounds were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 2 ml/kg b.wt. Groups of 20 to 30 animals were used. The treatment was administered p.o. 30 min before testing in individual cages. Palatable food intake was determined (weighed to the nearest 1 g) for a 30-min test session. Such test sessions were alternated with training sessions in which no treatment was administered but otherwise the test was carried out in the same manner as previously described. Evaluation of the results was first done with the Friedman two-way analysis of variance to demonstrate overall statistical significance. Whenever overall statistical significance was found, subsequent analysis was carried out with a two-tailed Wilcoxon test to compare the effect of each individual dosage condition for a test compound with that obtained with vehicle. A P value of ≤ .05 was accepted as statistically significant.

The minimal effective dose (MED) to produce a statistically significant reduction in palatable food intake was determined.

Schedule-induced polydipsia task in rats. Excessive drinking was induced in adult female RORO rats through the use of a fixed-time operant schedule (FT-1 min). The rats were drug-experienced and were food-deprived overnight before each test session. The test apparatus consisted of a sound-attenuated chamber surrounding a Plexiglas test box (30 × 25 × 30 cm) that was equipped with a stainless steel grid floor and a mechanism to permit the automatic delivery of one 45-mg food pellet (Formula A/I; P.J. Noyes Company, Inc., Lancaster, NH) each minute into a food cup. The test session was 1 h. The test compounds were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 2 ml/kg b.wt. In an initial experiment, either vehicle or a test compound was administered p.o. 30 min before the start of testing. The reference compound fluoxetine was tested at the doses 10, 30 and 60 mg/kg. Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 were tested at the doses 1, 3, and 10 mg/kg. A single group of 16 to 20 rats was used in each experiment to test vehicle and all of the selected doses of a given compound. Test days alternated with training days, on which the session proceeded in the same manner as on test days except that no treatment was given. In addition, we interspersed control sessions in which 60 food pellets were placed in the food cup at the start of the session and there was no other delivery of food during the entire 1-h test. A bottle containing tap water was always available on the test apparatus, but intake was measured (to the nearest 1 g) only for the test sessions and the control sessions. Evaluation of the results obtained in this test paradigm for both schedule-induced and control water intake was first done with the Friedman two-way analysis of variance to demonstrate overall statistical significance. Whenever overall statistical significance was found, subsequent analysis was carried out with a two-tailed Wilcoxon test to compare the effect of each individual dosage condition for a test compound to that obtained with vehicle. A P value of $\leq .05$ was accepted as statistically significant.

In a subsequent set of experiments using this polydipsia paradigm, an initial i.p. dose of either vehicle or the selective 5-HT$_{2C/2B}$ receptor antagonist SB 200646A at the dose 30 mg/kg (Kennett *et al.*, 1994) was administered 30 min before the test, followed 10 min later by p.o. administration of vehicle or the test compound (10 mg/kg Ro 60-0175/ORG 35030 or 20 mg/kg Ro 60-0332/ORG 35035). In a preliminary test it was shown that this dose of SB 200646A did not appreciably reduce stress-induced polydipsia. A single group of 14 to 16 rats was used in an experiment to evaluate each of these two test compounds. Testing then proceeded as previously described. Evaluation of the results obtained in this test paradigm was done using a two-tailed Wilcoxon test, a P value of $\leq .05$ being accepted as statistically significant.

Compulsive behavior in monkeys. Adult male squirrel monkeys first received p.o. administration of vehicle, fluoxetine (1, 3, 10 or 30 mg/kg), Ro 60-0175/ORG 35030 (0.3, 0.6, 1, 3 or 10 mg/kg) or Ro 60-0332/ORG 35035 (0.3, 1, 3 or 10 mg/kg). This dose of 8-OH-DPAT (0.1 mg/kg s.c.) was used because it was the lowest dose consistently to induce compulsive whole-body scratching ("displacement behavior") occurring periodically in bouts in squirrel monkeys (Moreau *et al.*, 1992). The test compounds were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 2 ml/kg b.wt. Groups of 4 to 5 monkeys per dose were used, and 14 monkeys were given vehicle treatment. Each monkey was tested only once in this entire experiment. Immediately after injection of 8-OH-DPAT, the monkeys were placed in individual test cages, and scratching bouts were scored over the 2-h observation period after injection. The observer was not blind with respect to the treatment condition. The ID_{50} values were calculated with probit analysis.

In a subsequent experiment, different groups of 10 monkeys received p.o. administration of vehicle, 1 mg/kg Ro 60-0175/ORG 35030 or 10 mg/kg fluoxetine once daily for 15 days. A challenge test with 8-OH-DPAT was done as described above on the first and the final treatment day to permit an assessment of tolerance development. Evaluation of the results was done using a two-tailed Mann-Whitney *U* test to compare treatment conditions and a two-tailed Wilcoxon test to compare the anticompulsive effect of each treatment condition on the first *vs.* the final treatment day. A P value of $\leq .05$ was accepted as statistically significant.

Passive avoidance deficit in olfactory-bulbectomized rats. Bulbectomized rats exhibit disturbed emotional behavior, which can be seen in impaired passive avoidance acquisition that is ameliorated by antidepressant treatment (Broekkamp *et al.*, 1980). Naive Sprague-Dawley rats underwent bilateral bulbectomy *via* a vacuum suction method under pentobarbital anesthesia (60 mg/kg i.p. Nembutal, supplemented as needed). During convalescence the operated rats were housed in groups of five in Macrolon®-type II cages (24 × 18 × 13 cm). Passive avoidance training began 10 days after surgery. The apparatus was a black plastic box (40 × 40 × 40 cm) with a transparent plastic lid and a stainless-steel grid floor. A plastic platform (40 × 8 cm) was attached to a wall 4 cm above the grid floor. Vehicle (physiological saline) or 5 mg/kg Ro 60-0332/ORG 35035 was injected s.c. 30 min before the start of testing. The volume administered was 5 ml/kg b.wt. A trial began by placing a rat on the elevated platform above an electrified grid (0.7-mA scrambled shock). When the rat stepped down onto the grid with all four paws, foot shock was delivered for 2 s. The stepdown latency for the first trial was recorded. After each trial the rat was returned to its home cage for a period of 1 min. Training continued until the rat stayed on the elevated platform for 5 min or for a maximum of 10 trials. Groups of 8 to 10 rats per treatment condition were used. The total trials required to meet the learning criterion was evaluated using a two-tailed Mann-Whitney *U* test to compare groups, a P value $\leq .05$ being accepted as significant. After the completion of testing, the rats were sacrificed, and the olfactory bulb ablations verified.

DRL-72 s task in rats. In a task employing the DRL-72 s, antidepressants have generally been found to dose-dependently decrease response rates but to increase the total number of reinforcements obtained in a session (O'Donnell and Seiden, 1983). Adult, male Long-Evans rats (325–425 g) were individually housed and maintained on a schedule of restricted feeding (15–20 g per day) with water available *ad libitum*. Testing took place in standard operant chambers (Med Associates, Inc., Georgia, VT) equipped with a house-light, two cue lamps placed directly over two levers and a centrally placed pellet dispenser with cue light. The testing schedule was controlled by a DEC 700 series interface connected to an IBM ESS 2 PC running MedState notation software. The daily test sessions lasted 1 h. In the DRL-72 s task, the rat must wait a minimum of 72 s between lever presses in order to obtain food reinforcement; an earlier response resets the clock and is not reinforced. Only the right-hand lever was active in this procedure. Rats underwent an intensive training schedule until stable responding was obtained (Andrews *et al.*, 1994), and evaluation of the test compounds followed. All rats were well trained and drug-experienced at the start of testing; difference groups of performance-matched rats were used to evaluate the different treatment conditions ($N = 10$–12). Vehicle (physiological saline/mulgofen), Ro 60-0175/ORG 35030 (1, 3 or 10 mg/kg), or Ro 60-0332/ORG 35035 (1, 3 or 10 mg/kg) was injected i.p. 30 min before the start of testing. The volume administered was 1 ml/kg b.wt. The total responses and total reinforcements per session were recorded. Data were analyzed with a one-factor ANOVA for independent groups, and *post-hoc* comparisons were performed with the Tukey multiple comparisons test using the Statistica software package (StatSoft Inc.), a P value $< .01$ being accepted as significant.

Sleep-wake behavior in rats. Sleep-wake stages were evaluated in rats using an automated system that records and analyzes bioelectrical signals arising from parieto-occipital EEG, mental EEG and a movement detector. Male Hsd/Cpb rats were anesthetized (60 mg/kg i.p. Nembutal, supplemented as needed) and stereotaxically implanted with two epidural screw electrodes over the right parieto-occipital cortex with an epidural grounding screw electrode implanted over the frontal cortex. Two Teflon-coated stainless steel

wire electrode were implanted bilaterally into the dorsal neck musculature and connected to screws fastened to the skull. After convalescence for at least 2 weeks, testing was done in a sound-attenuated box (100 × 40 × 200 cm) with the electrodes connected *via* a swivel to the data acquisition system. Gross movements were measured as capacitative artifacts generated in an open-ended wire in the flat cable between rat and swivel joint. Six sleep-wake stages are distinguished: 1) active waking characterized by movement, *theta* activity and high EMG, 2) quiet waking without movement, 3) quiet sleep, characterized by EEG spindles, 4) deep slow-wave sleep with prominent *delta* activity, 5) pre-REM sleep with spindles against a background of *theta* activity and low EMG and 6) REM sleep with *theta* activity and low EMG. The experimental compounds were given in physiological saline in a volume of 5 ml/kg b.wt. Treatment was given immediately before the start of testing. Fluoxetine was tested at the doses 1, 2.2, 3.2, 4.6, 10 and 32 mg/kg i.p. Ro 60-0175/ORG 35030 was tested at the doses 2.2 mg/kg i.p. and 3.2, 10 and 22 mg/kg p.o. Ro 60-0332/ORG 35035 was tested at the doses 1, 3.2 and 10 mg/kg i.p. and 3.2, 10 and 32 mg/kg p.o. Treatment was done at the start of the light cycle. Data for the initial 3 h after administration were evaluated. To reduce the variance in the analysis, percentage changes with respect to median values in the vehicle group were used. Statistical analysis was done with Student's *t* tests with a significance level set at $P < .05$. A washout period of 2 to 3 weeks between successive tests was allowed. Groups of 6 to 9 rats were used for each treatment condition. Additional methodological details are provided elsewhere (Ruigt *et al.*, 1989a,b).

Drugs

Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 were synthesized at F. Hoffmann-La Roche Ltd. (Basel, Switzerland) and are shown in figure 1. Fluoxetine HCl was purchased from Sigma Chemie (Buchs, St. Gallen, Switzerland). (±)-DOI HCl and m-CPP dihydrochloride were purchased from Research Biochemicals International (Natick, MA). SB200646A was synthesized at N.V. Organon. Ro 60-0491/001 and 8-OH-DPAT hydrochloride were synthesized at F. Hoffmann-La Roche Ltd. The conditions of administration are provided in the test descriptions. All doses were calculated for the salt.

Results

In Vitro Pharmacology

Receptor binding assays. The *in vitro* binding results for 5-HT receptors are shown for Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 in table 2. Ro 60-0175/ORG 35030 exhibits high-affinity binding to the human 5-HT_{2C} receptor (pK_i = 9.0) with affinity for several other 5-HT receptor subtypes (1A, 1B, 1D, 3, 4, 6, 7) found to be at least 3 logarithmic units lower; affinity to the human 5-HT_{2A} receptor was intermediate (pK_i = 7.5). Ro 60-0332/ORG 35035 exhibits high-affinity binding to human 5-HT_{2C} receptor (pK_i = 8.5) with affinity for several other 5-HT receptor subtypes (1A, 3, 4, 6, 7) found to be at least 2 logarithmic units lower; affinity to the human 5-$HT_{1D\alpha}$ receptor (pK_i = 7.4) and the human 5-HT_{2A} receptor (pK_i = 7.0) was intermediate. To assess the binding specificity, we performed a broad evaluation of an additional 26 receptors. IC_{50} values for Ro 60-0175/ORG 35030 were found to be >1 μM for all of the non-5-HT receptors listed in table 1 except the human *beta*-2 adrenoceptor (pIC_{50} = 6.6). IC_{50} values for Ro 60-0332/ORG 35035 were found to be >1 μM for all the non-5-HT receptors listed in table 1 except for rat dopamine D_3 receptor and the human muscarinic M_4 receptor, for which the approximate IC_{50} values were only slightly less than 1 μM.

TABLE 2
Binding affinity (pK_i) for 5-HT receptors

Receptor (species)	Ro 60-0175 (ORG 35030)	Ro 60-0332 (ORG 35035)
5-HT_{1A} (human)	5.4	5.7
5-HT_{1B} (rat)	5.3	n.d.
5-HT_{1D} (human)	5.7	7.4
5-HT_{2A} (human)	7.5	7.0
5-HT_{2C} (human)	9.0	8.5
5-HT_3 (rat)	5.2	<5
5-HT_4 (guinea pig)	5.7	n.d.
5-HT_6 (human)	5.2	6.2
5-HT_7 (human)	5.6	5.8

n.d. = not determined.

Stimulation of IP_3 formation. Both Ro 60-0175/ORG 35030 (pEC_{50} = 6.7, α = 1.1) and Ro 60-0332/ORG 35035 (pEC_{50} = 6.7, α = 1.0) exhibited a concentration-related increase in IP_3 formation in rat choroid plexus that achieved a maximal effect similar to that of 5-HT itself.

Behavioral observation in rats. Ro 60-0175/ORG 35030 (ED_{50} = 0.2 mg/kg s.c.), Ro 60-0332/ORG 35035 (ED_{50} = 1.9 mg/kg s.c.) and the reference compound m-CPP (ED_{50} = 0.3 mg/kg s.c.) induced penile erection in male rats (an effect that is only very infrequently observed in this test situation after vehicle treatment alone). The lowest doses to induce penile erection were 0.1 mg/kg Ro 60-0175/ORG 35030 (3/8 rats), 1 mg/kg Ro 60-0332/ORG 35035 (2/8 rats) and 0.1 mg/kg m-CPP (2/8 rats). A maximal effect was achieved for the two former compounds at approximately 3.2 mg/kg. These results on penile erection are illustrated in figure 2. Neither Ro

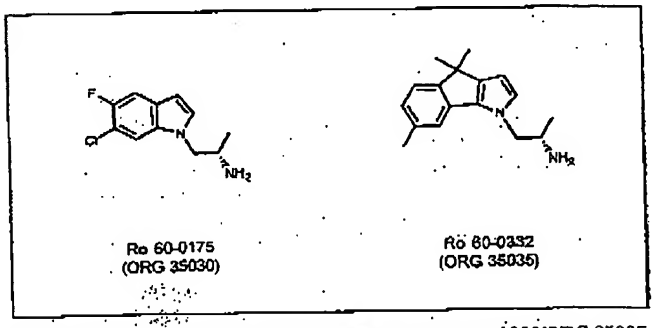

Fig. 1. Structures of Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035.

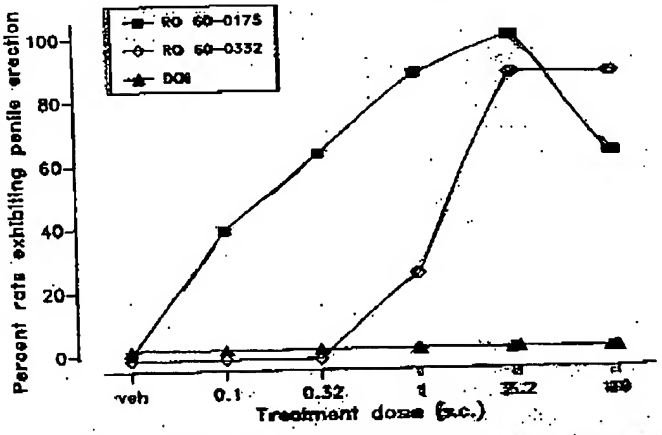

Fig. 2. Elicitation of penile erection in rats with (±)-DOI, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035.

60-0175/ORG 35030 nor Ro 60-0332/ORG 35035 elicited head shakes up to the dose 10 mg/kg (the highest dose tested). In contrast, p.o. administration of (±)-DOI failed to induce penile erection up to 10 mg/kg s.c. but dose-dependently increased head twitches, reaching a plateau effect of about 20 bouts (8/8 rats) at the dose 1 mg/kg and above (ED_{50} = 0.3 mg/kg s.c.). In a preliminary experiment it was shown that pretreatment with the 5-HT$_{2C}$ receptor antagonist Ro 60-0491 completely blocked the elicitation of penile erection by Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 (tested up to 10 mg/kg s.c.) without revealing any new behavioral symptoms—for example, head shakes.

Behavioral observation in squirrel monkeys. Observation of the effects produced by high p.o. doses of fluoxetine or the two experimental compounds was done in male monkeys. Vehicle treatment was used to define the base-line behavioral pattern. Compared with vehicle, the reference compound fluoxetine produced some signs of sedation in 2/10 and of retching and/or vomiting in 5/10 monkeys at 10 mg/kg; 30 mg/kg fluoxetine produced sedation in 5/10, retching and/or vomiting in 7/10, tremors in 2/10 and convulsions in 1/10 monkeys. Ro 60-0175/ORG 35030 induced signs of sedation in half of the monkeys at both 10 and 30 mg/kg, as well as retching and/or vomiting in 1/10 at 10 mg/kg and in only 2/10 at 30 mg/kg. Ro 60-0332/ORG 35035 induced some postural relaxation in 3/5 monkeys at 10 mg/kg, with signs of relaxation and/or sedation in 5/5 monkeys at 30 mg/kg; retching and/or vomiting was seen in only 2/5 monkeys at 30 mg/kg. Penile erection was not observed after any of the treatments in these male monkeys.

Spontaneous motor activity in rats. We evaluated the effect of prior injection of the 5-HT$_{2C}$ receptor antagonist Ro 60-0491 on the hypoactivity induced by a high dose of each of the 5-HT$_{2C}$ receptor agonists m-CPP (6 mg/kg i.p.), Ro 60-0175/ORG 35030 (10 mg/kg i.p.) and Ro 60-0332/ORG 35035 (10 mg/kg i.p.). The results are illustrated figure 3. When given after a vehicle injection, the selected dose of each of these three 5-HT$_{2C}$ receptor agonists significantly reduced total distance ($P < .05$) compared with vehicle plus vehicle treatment. (The selected dose of Ro 60-0491 given in combination with vehicle somewhat reduced locomotion in these separate experiments, but this reduction reached statistical significance only in the experiment with Ro 60-0332/ORG 35035). Of primary interest, however, was a determination of whether the antagonist Ro 60-0491 would attenuate the hypomotility induced by these 5-HT$_{2C}$ receptor agonists. In fact, Ro 60-0491 significantly reversed the hypoactivity induced by each of these three 5-HT$_{2C}$ receptor agonists ($P < .05$), although a full return to the base-line activity level was not obtained.

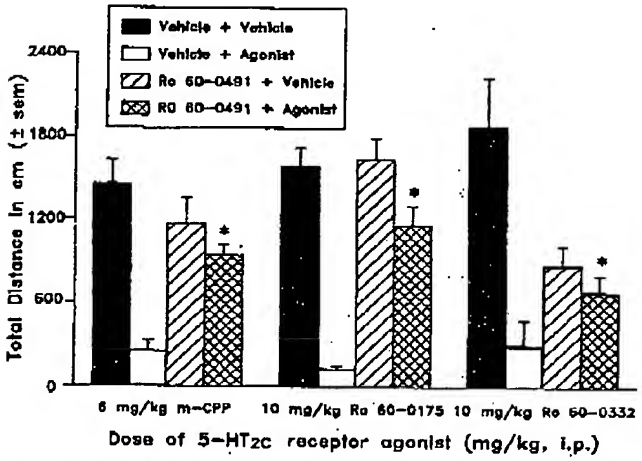

Fig. 3. Antagonism of the hypomotility in rats induced with m-CPP, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035. When given together with vehicle, each of these three 5-HT$_{2C}$ receptor agonists significantly reduced activity *vs.* the vehicle plus vehicle condition. (* $P < .05$ for comparison with group treated with vehicle and the 5-HT$_{2C}$ receptor agonist.) [Note: The selected dose of the 5-HT$_{2C}$ receptor antagonist Ro 60-0491 given together with vehicle significantly reduced activity compared to the vehicle plus vehicle condition only in the experiment with Ro 60-0332/ORG 35035 (significance not shown in figure).]

Anticonvulsant effects in mice. Ro 60-0175/ORG 35030 was anticonvulsant in the audiogenic seizure paradigm in DBA/2J mice with an ED_{50} of 56 mg/kg p.o. In contrast, Ro 60-0332/ORG 35035 protected maximally 25% of the mice against seizures at each of the doses 10, 32 and 100 mg/kg p.o.

Elevated plus-maze task in rats. A single injection of fluoxetine (5 mg/kg i.p.) significantly reduced, in comparison with vehicle, the number of open arms entered and the time spent in open arms ($P = .004$ and $P = .006$, respectively), which indicates an anxiogenic effect, but did not reduce the number of closed-arm entries or attempts; these results show that neither general motor activity nor risk assessment, respectively, was affected. Furthermore, the same pattern of effects was observed after the fifth in a series of daily treatments. In contrast, acute treatment with Ro 60-0175/ORG 35030 (1.5 mg/kg i.p.) failed to affect these same parameters significantly when compared with vehicle. This pattern of results was unchanged after repeated daily injections made on four successive days and followed on the final day by a test in the plus-maze. Ro 60-0332/ORG 35035 (7.5 mg/kg i.p.) did not affect the time spent in the open arms but significantly decreased the number of open-arm entries, closed-arm entries and attempts ($P = .02$, $P = .0001$ and $P = .02$, respectively). However, this pattern of results (especially reduced closed-arm entries) suggests general sensorimotor impairment rather than anxiogenesis. After the fifth in a series of daily treatments, none of these parameters was differentially affected by vehicle and Ro 60-0332/ORG 35035. The detailed results are provided in table 3.

Burying behavior in mice. The lowest dose of fluoxetine that was tested, 10 mg/kg s.c., reduced burying by 55%. The ED_{50} value for reducing burying behavior was 3.8 mg/kg s.c. for Ro 60-0175/ORG 35030 and 4.4 mg/kg s.c. for Ro 60-0332/ORG 35035.

Reduction of palatable food intake in rats. All of the test compounds were found to produce a statistically significant overall reduction in palatable food intake for the dose ranges evaluated ($P < .05$). Fluoxetine (MED = 60 mg/kg p.o.), m-CPP (MED = 0.3 mg/kg p.o.), Ro 60-0175/ORG 35030 (MED = 10 mg/kg p.o.) and Ro 60-0332/ORG 35035 (MED = 30 mg/kg p.o.) significantly reduced consumption ($P < .01$) in nondeprived rats during the 30-min session. At these MED values, the reduction in palatable food intake for these compounds was approximately 40%, except for m-CPP, where 0.3 mg/kg reduced intake by only 19% (but at 1 mg/kg, intake was reduced by 45%). These data are shown in figure 4.

TABLE 3
Effects of equieffective doses of fluoxetine, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 on behavioural parameters in a plus-maze task in rats

Treatment (i.p.)	Time (s) Spent in Open Arms	Open Arms Entered	Closed Arms Entered	Attempts
First Treatment Injection				
Vehicle	31.6 ± 5.9	3.3 ± 0.6	9.6 ± 0.6	6.2 ± 0.5
Fluoxetine	5.5 ± 2.1**	0.8 ± 0.2**	7.6 ± 1.0	5.6 ± 0.7
Ro 60-0175 (ORG 35030)	33.4 ± 9.1	3.7 ± 0.9	8.2 ± 0.8	4.9 ± 0.8
Ro 60-0332 (ORG 35035)	20.3 ± 7.0	1.4 ± 0.5*	4.4 ± 1.1***	3.8 ± 0.8*
Final Treatment Injection				
Vehicle	18.3 ± 6.9	1.7 ± 0.6	6.3 ± 1.3	4.6 ± 1.1
Fluoxetine	1.1 ± 0.7*	0.3 ± 0.2*	7.5 ± 1.3	4.9 ± 0.8
Ro 60-0175 (ORG 35030)	15.3 ± 6.3	1.8 ± 0.6	8.1 ± 1.8	4.5 ± 0.9
Ro 60-0332 (ORG 35035)	8.0 ± 5.1	0.7 ± 0.4	4.4 ± 1.0	3.5 ± 0.8

* $P < .05$; ** $P < .01$; *** $P < .001$ for comparison with vehicle condition.

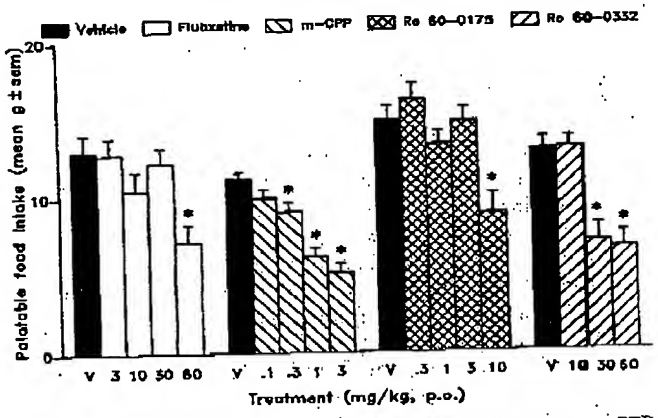

Fig. 4. Reduction of palatable food intake in rats by fluoxetine, m-CPP, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035. (* $P < .05$ for comparison with vehicle controls.)

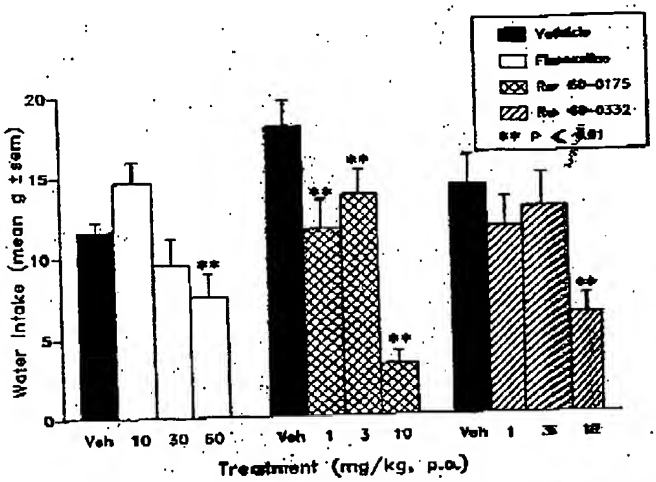

Fig. 6. Amelioration of schedule-induced polydipsia in rats by fluoxetine, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035. (** $P < .01$ for comparison with controls.)

Reversal of schedule-induced polydipsia task in rats. All of the test compounds were found to produce a statistically significant overall reduction in both schedule-induced drinking and control drinking (*i.e.*, under the non-stress condition) across the dose ranges evaluated ($P < .05$). Fluoxetine significantly reduced schedule-induced polydipsia at the high dose of 60 mg/kg p.o., whereas control water intake assessed in the same apparatus was already significantly reduced at 30 mg/kg p.o. In contrast, Ro 60-0175/ORG 35030 significantly reduced schedule-induced polydipsia beginning at 1 mg/kg p.o., a reduction in control water intake first being observed at 10 mg/kg. Ro 60-0332/ORG 35035 significantly decreased both schedule-induced polydipsia and control water intake at 10 mg/kg. These results are shown in figure 5.

A subsequent experiment was done using the 5-$HT_{2C/2B}$ receptor antagonist SB 200646A. In combination with a vehicle pretreatment, the selected dose of Ro 60-0175/ORG 35030 (10 mg/kg p.o.) and that of Ro 60-0332/ORG 35035 (20 mg/kg p.o.) significantly reduced schedule-induced polydipsia in comparison with a vehicle plus vehicle condition ($P < .01$). Pretreatment with SB 200646A (30 mg/kg i.p.) significantly reduced the schedule-induced polydipsia produced by either of these 5-HT_{2C} receptor agonists ($P < .01$) compared with pretreatment with vehicle. These results are shown in figure 6.

Reduction of compulsive behavior in monkeys. Compulsive whole-body scratching bouts are induced in squirrel monkeys by 8-OH-DPAT, and the effect can be fully reversed by subsequent treatment with 5-HT_{2C} receptor agonists. This provides a robust pharmacodynamic model in monkeys. 8-OH-DPAT produced multiple scratching bouts throughout the 2-h observation period when it was given after vehicle (mean ± S.E. = 86.2 ± 11.4). Compared with vehicle, fluoxetine reduced scratching only beginning at 10 mg/kg (about 26% reduction), with 30 mg/kg (the highest dose tested) producing an only somewhat greater effect (37% reduction). In contrast, both Ro 60-0175/ORG 35030 (ID_{50} = 0.8 mg/kg) and Ro 60-0332/ORG 35035 (ID_{50} = 1.1 mg/kg) potently reduced scratching, achieving a full reversal at the dose 3 mg/kg. These results are illustrated in figure 7. In comparison, the nonselective 5-HT_{2C} receptor agonist m-CPP had an ID_{50} value of 0.3 mg/kg p.o. in this test paradigm (data not included in fig. 7). In the chronic experiment, the anticompulsive effects of 1 mg/kg p.o. Ro 60-0175/ORG 35030 and 10 mg/kg p.o. fluoxetine were of approximately the same magn-

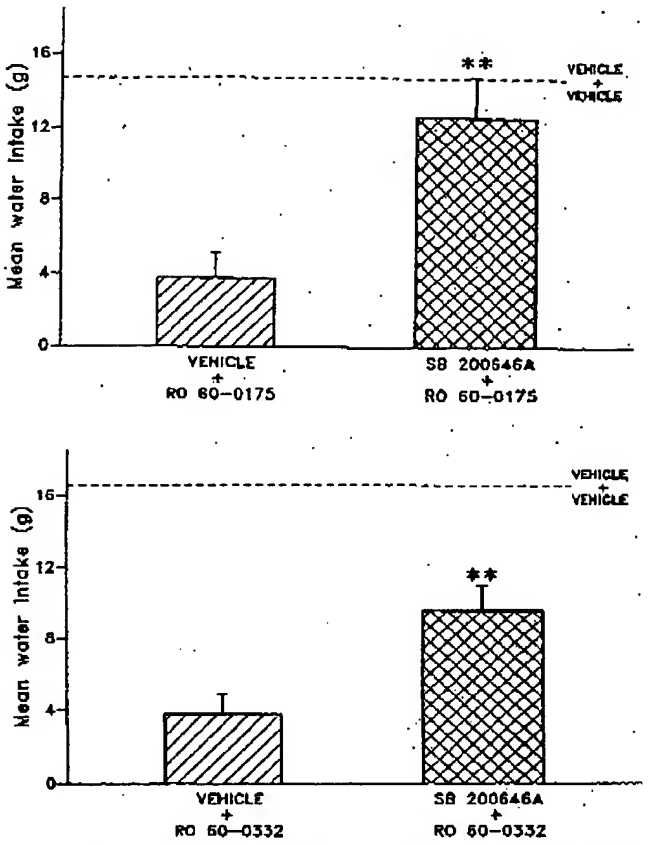

Fig. 6. Pretreatment with 5-HT$_{2C/2B}$ receptor antagonist SB 200646A decreases the ameliorative effect of Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 in schedule-induced polydipsia paradigm. (** $P < .01$ for comparison with vehicle controls.)

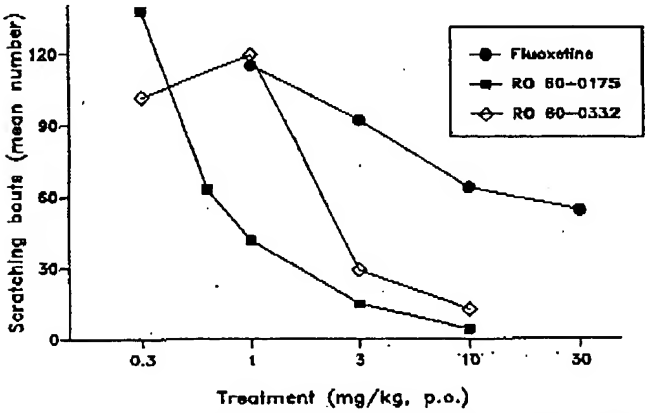

Fig. 7. Reduction of 8-OH-DPAT-induced scratching in squirrel monkeys with fluoxetine, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 (in these groups, S.E. values were typically about 20–35% of the mean). After vehicle treatment, 8-OH-DPAT induced a mean (± S.E.) of 86.2 ± 11.4 scratching bouts during the 2-h observation period.

nitude, with a significant difference from vehicle treatment ($P < .05$). There was no difference observed between the first and the final day for either of these treatments, which indicates that tolerance had not developed.

Reversal of passiv avoidance deficit in olfactory-bulbectomized rats. Compared with sham-operated control rats receiving vehicle, the rats with verified bilateral olfactory bulbectomies given vehicle required significantly more trials to reach the learning criterion in a passive avoidance task ($P < .05$). Treatment with Ro 60-0332/ORG 35035 (5 mg/kg s.c.) in bulbectomized rats significantly reduced the number of trials to acquisition compared with vehicle ($P < .05$). These results are shown in figure 8. The step-down latency on the first trial (before any shock exposure) was comparable in the sham-operated, vehicle-treated group (10.9 ± 2.6 s) and the bulbectomized group receiving Ro 60-0332/ORG 35035 (9.4 ± 1.2 s), whereas that of the bulbectomized, vehicle-treated group was lower (4.6 ± 0.6 sec).

Responding in DRL-72 s task in rats. Both Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 significantly decreased the rate of lever pressing only at 10 mg/kg i.p. ($P < .01$) with no accompanying signs of observable sedation. However, at the highest dose that failed to reduce total number of responses in a session (3 mg/kg i.p.), Ro 60-0175/ORG 35030 significantly increased the number of reinforcements obtained during the session (2.6-fold vehicle base line; $P < .01$), and Ro 60-0332/ORG 35035 nonsignificantly increased this parameter (1.7-fold vehicle base line).

Sleep-wake pattern in rats. It has previously been shown that in the EEG-defined rat sleep-wake pattern, an increase in duration of the quiet-waking component, accompanied by a decrease in the REM component, was typical for the general class of antidepressants (Ruigt *et al.*, 1993). Fluoxetine was found to increase quiet waking appreciably and to decrease REM sleep at 10 and 32 mg/kg i.p. Ro 60-0175/ORG 35030 produced a similar pattern of effects at 2.2 mg/kg i.p. and at 10 and 22 mg/kg p.o. Ro 60-0332/ORG 35035 showed this typical antidepressant profile at 3.2 and 10 mg/kg i.p. and at 32 mg/kg p.o. These results are summarized in table 4.

Discussion

Serotonin neurotransmission plays an important role in numerous physiological processes both in health and in psychiatric disorders. Determination of the functions of the mul-

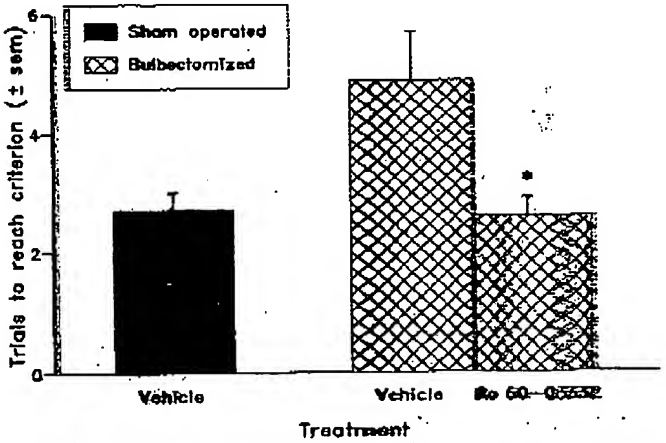

Fig. 8. Amelioration of passive avoidance deficit in olfactory bulbectomized rats with Ro 60-0332/ORG 35035. (* $P < .05$ for comparison with vehicle-treated olfactory bulbectomized rats.)

TABLE 4
Pharmaco-EEG evaluation in free-moving rats (the results are shown as percentage of base-line duration)

Compound	Dose (mg/kg) and Route (N)	Quiet Waking	REM Sleep
Fluoxetine	1 i.p. (8)	2	−45
	2.2 i.p. (8)	−17	6
	3.2 i.p. (6)	−8	−87
	4.6 i.p. (9)	−4	−51
	10 i.p. (8)	55	−100*
	32 i.p. (7)	126*	−81*
Ro 60-0175 (ORG 35030)	2.2 i.p. (7)	94*	−81
	3.2 p.o. (8)	6	−61
	10 p.o. (8)	63	−100*
	22 p.o. (7)	96*	−100*
Ro 60-0332 (ORG 35085)	1 i.p. (8)	19	−8
	3.2 i.p. (7)	84*	−47*
	10 i.p. (7)	370*	−100*
	3.2 p.o. (7)	30	−64
	10 p.o. (8)	76	43
	32 p.o. (5)	122*	−65*

Note: * $P < .05$ for comparison with vehicle control.

tiple 5-HT receptor subtypes identified in recent years has been dependent on the availability of selective agonists and antagonists. The present study focused on investigating the putative involvement of 5-HT_{2C} agonism in psychiatric disorders by testing the 5-HT receptor agonists Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 in animal models of compulsive behavior, depression and anxiety.

A broad evaluation of receptor binding affinity was carried out for 35 different receptors. It was demonstrated that both Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 exhibited high affinity for the human 5-HT_{2C} receptor (pK_i = 9.0 and 8.5, respectively) and considerably lower affinity for the human 5-HT_{2A} receptor (pK_i = 7.5 and 7.0, respectively). Affinity of these two compounds for numerous other selected receptors was at least 2 to 3 logarithmic units weaker than for the 5-HT_{2C} receptor. Both of these experimental compounds produced a concentration-dependent stimulation of IP_3 formation in rat choroid plexus *in vitro* in a manner similar to 5-HT itself, an effect that demonstrates their 5-HT_{2C} receptor agonism. In an experiment using NIH 3T3 cells expressing either h5-HT_{2C} or h5-HT_{2A} receptors, EC_{50} values for stimulation of IP_3 formation were also determined: Ro 60-0175/ORG 35030 (2C: 287 nM; 2A: 671 nM), Ro 60-0332/ORG 35035 (2C: 315 nM; 2A: 1496 nM) and reference compound m-CPP (2C: 357 nM; 2A: 1384 nM). Under this set of test conditions, selectivity for h5-HT_{2C} *vs.* h5-HT_{2A} receptors was about 2- to 5-fold (unpublished data). Both experimental compounds elicited penile erection in rats, a behavioral sign of the serotonin syndrome in rodents, which is indicative of a 5-HT_{2C} agonistic profile (Berendsen *et al.*, 1990; Millan *et al.*, 1997); the virtual absence of concomitant head shakes is a clear indication of the absence of any appreciable 5-HT_{2A} receptor agonism in the *in vivo* situation (it is interesting to note that SSRIs also elicit penile erections in rats; see Berendsen, 1995).

Results from the isolated rat fundus strip assay indicate that Ro 60-0175/ORG 35030 (pD_2 = 7.8, 8.0, with a maximal response of only 70% of that obtained with 5-CT) and Ro 60-0332/ORG 35035 (pD_2 = 6.1) act as agonists at the 5-HT_{2B} receptor (unpublished data). These results, together with data on the potencies of these two compounds for stimulating IP_3 formation *in vitro* in tissue and NIH 3T3 cells, suggest that there may be at most only minimal selectivity for 5-HT_{2C} *vs.* 5-HT_{2B} receptors. Little is currently known about the function of 5-HT_{2B} receptors, in part because of the lack of highly selective ligands. It has been reported that this receptor is found in the GI tract, the heart and possibly the brain (Baxter *et al.*, 1995; Duxon *et al.*, 1995; Loric *et al.*, 1992), as well as in the meninges (Schmuck *et al.*, 1996). It has been reported that the preferential 5-HT_{2B} agonist BW723C86 exhibits both anxiolytic and hyperphagic effects in rats (Ainsworth *et al.*, 1996; Kennett *et al.*, 1996). At the present time, however, it is difficult to define the possible contribution of 5-HT_{2B} receptor agonism to the pharmacological profiles of Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035; this should receive attention in further studies.

Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 were well tolerated in squirrel monkeys, signs of sedation being the most commonly observed side effect at high doses. Tests with a number of structurally different 5-HT_{2C} receptor agonists, including these two compounds, consistently produced penile erection in rats but not in squirrel monkeys. In addition, these two compounds were shown to be weakly anticonvulsant in mice, which, in view of the high doses required, is probably not related to their 5-HT_{2C} receptor agonism. Hypomotility induced in rats by a high dose of either of these two compounds was significantly attenuated by pretreatment with the 5-HT_{2C} receptor antagonist Ro 60-0491. In the elevated plus-maze task in rats, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 failed to exhibit either an anxiogenic effect or an anxiolytic effect (whereas fluoxetine was anxiogenic).

In the burying test in mice, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 were effective in significantly reducing this compulsive burying of novel objects. Similarly, excessive consumption of palatable food by nondeprived rats was significantly decreased by these two compounds, as previously reported for nonselective 5-HT_{2C} receptor agonists in tests of food consumption (Kennett, 1993). However, this may represent an anorectic effect rather than attenuation of compulsive responding *per se*. Schedule-induced polydipsia in rats has been proposed, on the basis of pharmacological validation, as an animal model of OCD (Wood *et al.*, 1995). Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 significantly attenuated excessive drinking in this task, a result that suggests their possible therapeutic value in OCD (fluoxetine was less potent and exhibited a weaker effect up to the highest tolerated dose). The ameliorative activity of both compounds could be reversed by pretreatment with the 5-$HT_{2C/2B}$ receptor antagonist SB 200646A. However, in this same experimental paradigm the preferential 5-HT_{2A} receptor antagonist ketanserin tartrate (30 mg/kg i.p.) was ineffective in reversing the ameliorative effect of either of these two compounds (unpublished data). In squirrel monkeys, 8-OH-DPAT injection elicits excessive whole-body scratching that lasts for several hours. Pretreatment with Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 potently and fully prevented this excessive behavior, which, in view of the low affinity for the 5-HT_{1A} receptor, is probably due to an inhibitory interaction with 5-HT_{2C} receptors (Berendsen *et al.*, 1990; Jenck *et al.*, 1994). Furthermore, there was no tolerance to this pharmacological effect over a 2-week treatment

period with either Ro 60-0175/ORG 35030 or fluoxetine. Interestingly, fluoxetine (which, along with other SSRRs, is approved for the therapy of OCD) was effective in attenuating excessive behavior in these diverse animal tests. However, either the potency of fluoxetine was lower than that of Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 or the effect size was smaller for fluoxetine (or both).

Antidepressants have been demonstrated to ameliorate the impairment in passive avoidance learning induced by olfactory bulbectomy in rats (Broekkamp *et al.*, 1980). In the present investigation, Ro 60-0332/ORG 35035 also significantly reduced the deficit in bulbectomized rats. In addition, Ro 60-0175/ORG 35030 significantly, and Ro 60-0332/ORG 35035 nonsignificantly, increased the total number of reinforcements obtained by rats in the DRL-72 s task, an effect that is suggestive of possible antidepressant activity (O'Donnell and Seiden, 1983). As further evidence of possible therapeutic potential of 5-HT$_{2C}$ receptor agonists in depression, it was shown that in a pharmaco-EEG paradigm in free-moving rats, treatment with fluoxetine, Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 appreciably increased the quiet-waking component and decreased REM sleep, an EEG profile typical of antidepressants (Kupfer *et al.*, 1981). Such results are consistent with these compounds' effectiveness in ameliorating mild stress-induced anhedonia in rats (Moreau *et al.*, 1996).

In conclusion, using the 5-HT$_{2C}$ receptor agonists Ro 60-0175/ORG 35030 and Ro 60-0332/ORG 35035 as research tools, it was possible to demonstrate both their excellent tolerability and their therapeutic potential for OCD and depression. Recently reported evidence for the effectiveness of both of these compounds in preventing and in curing mild stress-induced anhedonia and attenuating experimentally induced panic in rats further substantiates the therapeutic potential of 5-HT$_{2C}$ receptor agonists in psychiatry (Jenck *et al.*, in press; Moreau *et al.*, 1996).

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References

Abramowski D, Rigo M, Duc D, Hoyer D and Staufenbiet M (1995) Localization of the 5-hydroxytryptamine2C receptor protein in human and rat brain using specific antisera. *Neuropharmacology* 34:1635–1645.

Ahlenius SN, Fernandez-Guasti A, Hjorth S and Larsson K (1986) Suppression of lordosis behavior by the putative 5-HT receptor agonist 8-OH-DPAT in the rat. *Eur J Pharmacol* 124:361–363.

Ainsworth K, Trail B, Blackburn TP, Baxter GS and Kennett GA (1996) Is BW 723C86-induced hyperphagia an *in vivo* model of rat central 5-HT$_{2B}$ receptor function? *Br J Pharmacol* 117:suppl 178P.

Andrews JS, Jansen JHM, Linders S, Princen A, Drinkenburg WHIM, Coenders CJH and Vossen JHM (1994) Effects of imipramine and mirtazepine on operant performance in the rat. *Drug Dev Res* 32:58–66.

Baxter G, Kennett G, Blaney F and Blackburn T (1995) 5-HT$_2$ receptor subtypes: A family re-united? *TIPS* 16:105–110.

Berendsen HHG and Broekkamp CLE (1994) Comparison of stimulus properties of fluoxetine and 5HT receptor agonists in a conditioned taste aversion procedure. *Eur J Pharmacol* 253:83–89.

Berendsen HHG (1995) Interactions between 5-hydroxytryptamine receptor subtypes: Is a disturbed receptor balance contributing to the symptomatology of depression in humans? *Pharmacol Ther* 66:17–37.

Berendsen HHG and Gower AJ (1986) Opiate-androgen interactions in drug-induced yawning and penile erections in the rat. *Neuroendocrinology* 42:186–190.

Berendsen HHG, Jenck F and Broekkamp CLE (1990) Involvement of 5-HT$_{1C}$ receptors in drug-induced penile erections in rats. *Psychopharmacology* 101:57–61.

Bourson A, Wanner D, Wyler R, Petit M, Zwingelstein C, Rudler A and Sleight AJ (1995) Pharmacological evaluation of the discriminative stimulus of metachlorophenylpiperazine. *Pharmacol Biochem Behav* 52:107–114.

Broekkamp CLE and Berendsen HHG (1992) The importance of 5-HT$_{1C}$-receptors for anti-depressant effects. *Pol J Pharmacol Pharm* 44:suppl 20.

Broekkamp CLE, Garrigou D and Lloyd KG (1980) Serotonin-mimetic and antidepressant drugs on passive avoidance learning by olfactory bulbectomized rats. *Pharmacol Biochem Behav* 13:649–656.

Den Boer JA and Westenberg HGM (1995) Serotonergic compounds in panic disorder, obsessive-compulsive disorder and anxious depression: A concise review. *Human Psychopharmacol* 10:S173–S183.

Duxon MS, Reaveley AC, Flanigan TP, Baxter G, Blackburn TP and Fone KCF (1995) Expression of the 5-HT$_{2B}$ receptor protein in the rat brain. *Br J Pharmacol* 115:105P.

Goa KL and Ward A (1986) Buspirone: A preliminary review of its pharmacological properties and therapeutic efficacy as an anxiolytic. *Drugs* 32:114–129.

Jenck F, Moreau J-L, Berendsen HG, Ellis M, Broekkamp CLE, Martin JR, Wichmann J and Van Delft AML (1998) Antiaversive effects of 5HT2C receptor agonists and fluoxetine in a model of panic-like anxiety in rats. *Eur Neuropsychopharmacol*, in press.

Jenck F, Moreau J-L, Mutel V and Martin JR (1994) Brain 5-HT$_{1C}$ receptors and antidepressants. *Prog Neuro-Psychopharmacol Biol Psychiat* 18:563–574.

Kahn RS and Wetzler S (1991) m-Chlorophenylpiperazine as a probe of serotonin function. *Biol Psychiatry* 30:1139–1165.

Kennedy SH and Goldbloom DS (1994) Advances in the treatment of anorexia nervosa and bulimia nervosa. *CNS Drugs* 1:201–212.

Kennett GA (1993) 5-HT1C receptors and their therapeutic relevance. *Curr Opin Invest Drugs* 2:317–352.

Kennett GA, Bright F, Trail B, Baxter GS and Blackburn TP (1996) Effects of the 5-HT$_{2B}$ receptor agonist, BW 723C86, on three rat models of anxiety. *Br J Pharmacol* 117:1443–1448.

Kennett GA, Wood MD, Glen A, Grewall S, Forbes I, Gadre A and Blackburn TP (1994) *In vivo* properties of SB 200646A, 5-HT$_{2C/2B}$ receptor antagonist. *Br J Pharmacol* 111:797–802.

Kupfer DJ, Spiker DG, Coble PA, Neil JF, Ulrich R and Shaw DH (1981) Sleep and treatment prediction in endogenous depression. *Am J Psychiat* 138:429–434.

Leander JD (1987) Fluoxetine suppressed palatability-induced ingestion. *Psychopharmacology* 91:285–287.

Loi V, Lai M, Pisano MR and Del Zompo M (1995) Panicogenic effect of sumatriptan: A pilot study. *Pharmacol Res* 31:suppl 245.

Loric S, Launay J-M, Colas JF and Maroteaux L (1992) New mouse 5-HT$_2$-like receptor. Expression in brain, heart and intestine. *FEBS Letters* 312:203–207.

Lucki K, Kreider MS and Simanaky KJ (1988) Reduction of feeding behavior by the serotonin uptake inhibitor sertraline. *Psychopharmacology* 96:289–295.

Maswood S, Andrade M, Caldarolé-Pastemaka M and Uphouse L (1996) Protective actions of the 5-HT$_{2A/2C}$ receptor agonist, DOI, on 5-HT$_{1A}$ receptor mediated inhibition of lordosis behavior. *Neuropharmacology* 35:497–501.

McPherson GA (1985) KINETIC, EBCA, LIGAND, LOWRY: A collection of radioligand binding analysis programmes. Elsevier, Cambridge, MA.

Milne RJ and Heel RC (1991) Ondansetron: Therapeutic use as an antiemetic. *Drugs* 41:574–595.

Moreau J-L, Bös M, Jenck F, Martin JR, Mortas P and Wichmann J (1996) 5-HT$_{2C}$ receptor agonists exhibit antidepressant-like properties in the anhedonia model of depression in rats. *Eur Neuropsychopharmacol* 6:169–175.

Moreau JL, Griebel G, Jenck F, Martin JR, Widmer U and Haefely WE (1992) Behavioral profile of the 5HT$_{1A}$ receptor antagonist (S)-UH-301 in rodents and monkeys. *Brain Res Bull* 29:901–904.

Moreau J-L, Jenck F, Martin JR, Perrin S and Haefely WE (1993) Effects of repeated mild stress and two antidepressant treatments on the behavioral responses to 5HT$_{2C}$ receptor activation in rats. *Psychopharmacology* 110:140–144.

Moser PC, Tricklebank MD, Middlemiss DN, Mir AK, Hibert MF and Fozard JR (1990) Characterization of MDL 73005EF as a 5-HT$_{1A}$-selective ligand and its effects in animal models of anxiety: Comparison with buspirone, 8-OH-DPAT and diazepam. *Br J Pharmacol* 99:343–349.

Munson PJ and Rodbard D (1980) LIGAND: A versatile computerized approach for characterisation of ligand-binding systems. *Anal Biochem* 107:220–239.

O'Donnell JM and Seiden LS (1983) Differential-reinforcement-of-low-rate 72-sec schedule: Selective effects of antidepressant drugs. *J Pharmacol Exp Ther* 227:80–88.

Pompeiano M, Palacios JM and Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family RNAs: Comparison between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. *Mol Brain Res* 23:163–178.

Rasmussen SA, Eisen JL and Pato MT (1993) Current issues in the pharmacologic management of obsessive compulsive disorder. *J Clin Psychiat* 54:suppl 4–53.

Ruigt GSF, Engelen S, Gemits A and Verlaan F (1992) Computer-based predictions of psychotropic drug classes based on a discriminant analysis of drug effects on rat sleep. *Neuropsychobiology* 28:138–153.

Ruigt GSF, Van Proosdij JN and Van Delft AML (1989a) A large scale, high resolution, automated system for rat sleep staging. I. Methodology and technical aspects. *Electroencephalogr Clin Neurophysiol* 73:52–63.

Ruigt GSF, Van Proosdij JN and Van Wezenbeek LACM (1989b) A large scale, high resolution, automated system for rat sleep staging. II. Validation and application. *Electroencephalogr Clin Neurophysiol* 73:65–71.

Saxena PR (1995) Serotonin receptors: Subtypes, functional responses and therapeutic relevance. *Pharmacol Ther* 66:339–368.
Schmuck K, Ullmer C, Kalkman HO, Probst A and Lübbert H (1996) Activation of meningeal 5-HT_{2B} receptors: An early step in the generation of migraine headache. *Eur J Neurosci* 8:959–967.
Sleight AJ, Stam NJ, Mutel V and Vanderheyden PML (1996) Radiolabelling of the human 5-HT_{2A} receptor with an agonist, a partial agonist and an antagonist: Effects on apparent agonist affinities. *Biochem Pharmacol* 51:71–76.
Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF and Julius D (1995) Eating disorder and epilepsy in mice lacking 5-HT_{2C} serotonin receptors. *Nature (Lond)* 374:542–546.
Wood A, Smith C, Szewczak M, Dunn RW, Cornfeldt M and Corbett R (1993) Selective serotonin re-uptake inhibitors decrease schedule-induced polydipsia in rats: A potential model for obsessive compulsive disorder. *Psychopharmacology* 112:195–198.
Zohar J and Cohen R (1995) Serotonin function in OCD: Implications for treatment. Abstract book of the Second International Conference on New Directions in Affective Disorders, Jerusalem, September 3–8 (Abstract No. S 253).

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Novel Agonists of $5HT_{2C}$ Receptors. Synthesis and Biological Evaluation of Substituted 2-(Indol-1-yl)-1-methylethylamines and 2-(Indeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamines. Improved Therapeutics for Obsessive Compulsive Disorder†

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The syntheses of a series of substituted 2-(indol-1-yl)-1-methylethylamines and 2-(indeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamines are reported. The binding affinities of the compounds at $5HT_{2C}$ and $5HT_{2A}$ receptors (79% homology in the transmembrane domain) were determined. The ligands displayed selectivity for $5HT_{2C}$ receptors relative to $5HT_{2A}$ receptors. Compounds were functionally characterized both *in vitro* and *in vivo* as $5HT_{2C}$ receptor agonists. **5f**, **5l**, **5n**, **5o**, **5q**, **14c**, **14f**, **14k**, and **14m** exhibited anticompulsive activity in an animal model of obsessive compulsive disorder.

Introduction

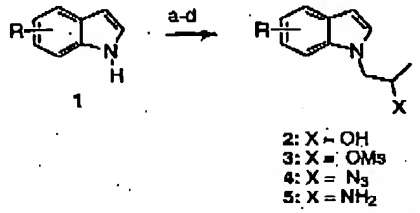
Selective serotonin reuptake inhibitors (SSRIs) increase extracellular levels of serotonin (5HT) and thereby nonselectively cause stimulation of all postsynaptic 5HT receptor subtypes. SSRIs have become standard therapy for neuropsychiatric disorders such as obsessive compulsive disorder (OCD), depression, and panic anxiety. There is accumulating evidence for the involvement of $5HT_{2C}$ receptor-mediated functions in the therapeutic efficacy of SSRIs.[1,2] The increased 5HT synaptic content resulting from the reuptake inhibition also allows 5HT to act on the other 5HT receptor subtypes, possibly explaining some of the side effects associated with SSRI treatment. Selective $5HT_{2C}$ receptor agonists, therefore, may represent a direct means to produce the beneficial therapeutic effects of SSRIs without concomitant side effects.

Our goal was to find $5HT_{2C}$ receptor agonists which (i) display at least 10-fold selectivity versus the $5HT_{2A}$ receptor subtype, for which sequence homology of the transmembrane region is high, (ii) show *in vivo* activity after oral administration in functional models of $5HT_{2C}$ receptor activation, and (iii) demonstrate therapeutic potential in an animal model of obsessive compulsive disorder.

Glennon et al. have shown that *N,N*-dimethylisotryptamines, i.e. derivatives of *N,N*-dimethyl-2-(indol-1-yl)ethylamines are isosteric with the corresponding *N,N*-dimethyltryptamines with respect to serotonin receptor affinity.[3] Such compounds are readily available via N-alkylation. We therefore screened isotryptamines for $5HT_{2C}$ receptor affinity and extended our study to the methylene homologues 1,4-dihydroindeno[1,2-*b*]pyrroles.

In this paper we report on the synthesis and the pharmacology of indoles and 1,4-dihydroindeno[1,2-*b*]-pyrroles in which a 2-aminopropyl side chain is attached to the N atom of the heterocycle. In analogy to phenylalkylamines, the α-methyl group was incorporated in order to suppress metabolic side chain deamination and to increase the lipophilicity of the compounds, allowing better CNS penetration.[4] Within these series of compounds we have identified agonists to the $5HT_{2C}$ receptor binding with high affinity and selectivity versus the $5HT_{2A}$ receptor. Some of these new ligands were evaluated in rats in the schedule-induced polydipsia paradigm, an animal model of obsessive compulsive disorder.[5] As a comparison we have included 5-fluoro-α-tryptamine (**15**) [6] and fluoxetine in our study.

Scheme 1[a]



[a] (a) Propylene oxide, NaH, THF; (b) MsCl, NEt_3, CH_2Cl_2; (c) NaN_3, DMF; (d) PtO_2, H_2, EtOH.

Chemistry

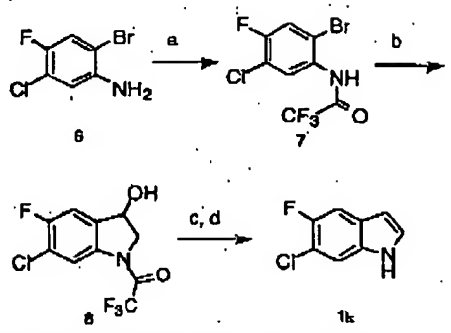
Substituted 2-(indol-1-yl)-1-methylethylamines **5** were prepared according to Scheme 1. Deprotonation of indoles **1** followed by alkylation with propylene oxide led to the secondary alcohols **2**. S_N2 reaction of the corresponding mesylates **3** with sodium azide and reduction of the azides **4** with either hydrogen or $LiAlH_4$ produced the amines **5** with excellent yields. The enantiomerically pure compounds **5k**−**q** were prepared from the (*R*)- or (*S*)-epoxide with inversion of configuration at the stereogenic center. The monosubstituted indoles are commercially available.

The dihalogenated building blocks can be prepared from the corresponding dihalogenated nitrotoluenes as described in the patent literature.[7] For the synthesis of 5-chloro-6-fluoroindole **1k** we have adopted a protocol developed by Wender and White[8] (Scheme 2). 2-Bromo-4-chloro-3-fluorophenylamine (**6**)[9] was acylated with trifluoroacetic anhydride to give **7**. Upon treatment with methyllithium and *tert*-butyllithium, a dilithium

† Dedicated to Prof. Dr. Dieter Seebach on the occasion of his 60th birthday.

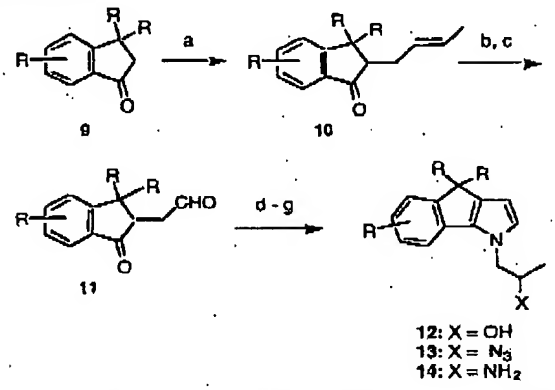
[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.

Scheme 2[a]



[a] (a) $(CF_3CO)_2O$, Na_2CO_3, diethyl ether; (b) MeLi, *t*-BuLi, −100 °C, chloroacetaldehyde, THF; (c) *p*-TsA, toluene; (d) NaOH, MeOH.

Scheme 3[a]



[a] (a) 3-Buten-2-ol, *p*-TsA, 2,2-dimethoxypropane; (b) ozone, CH_2Cl_2–MeOH; (c) TFA, CH_2Cl_2; (d) 1-amino-2-propanol, *p*-TsA, toluene; (e) MsCl, NEt_3, CH_2Cl_2; (f) NaN_3, DMF; (g) PtO_2, H_2, EtOH.

reagent was formed which underwent cyclization with chloroacetaldehyde[10] to the hydroxyamide **8**. Dehydration followed by hydrolysis gave **1k** in nine steps and an overall yield of 15%.

The preparation of the substituted 1,4-dihydroindeno-[1,2-*b*]pyrroles is shown in Scheme 3. Alkylation of the indan-1-ones **9** was performed by Claisen rearrangement of an *in situ* formed allyl vinyl ether system. Ozonolysis of **10** and subsequent cleavage of the acetal with TFA led to the 1,4-dicarbonyl compounds **11**, which were then reacted with commercially available 1-amino-2-propanol [(*S*), (*R*), (*RS*)] to yield the 1,4-dihydroindeno-[1,2-*b*]pyrroles **12**. The secondary alcohols were transformed into the amines **14** via the azides **13** as described for the synthesis of the indole derivatives **5**.

Pharmacology

The affinity of the compounds for $5HT_{2C}$ and $5HT_{2A}$ human receptors was assessed using displacement of [^{3}H]5HT and [^{3}H]DOB, respectively. To assess functional efficacy at $5HT_{2C}$ receptors, the ligands were evaluated for stimulation of phosphoinositol turnover in the choroid plexus of the rat. The compounds were also assessed for induction of penile erection in rats which is a symptom of the serotonin syndrome reflecting $5HT_{2C}$ receptor activation in rodents.[11] Finally, compounds which displayed interesting *in vivo* activity were further tested in the schedule-induced polydipsia model of OCD in rats for potential anticompulsive effects.[5]

Table 1. Substituents, Binding Affinities (pK_i) for $5HT_{2A}$ and $5HT_{2C}$ Receptors, Efficacy (pEC_{50} and Intrinsic Activity) in Inducing IP_3 Formation *in Vitro*, and Selectivity Ratio $5HT_{2C}$:$5HT_{2A}$ for **5**, **14**, and **15**

		pK_i		IP_3 formation		
compd	R	$5HT_{2A}$	$5HT_{2C}$	pEC_{50}	intrinsic activity	ratio
5a(*R,S*)	5-OMe	not tested	6.1 ± 0.03	5.0	0.8	
5b(*R,S*)	4- OMe	not tested	6.9 ± 0.08	4.9	0.8	
5c(*R,S*)	4-Me	7.0 ± 0.01	8.2 ± 0.08	5.6	0.6	16
5d(*R,S*)	4-F	6.8 ± 0.06	8.1 ± 0.03	5.9	0.7	20
5e(*R,S*)	5-Me	6.1 ± 0.01	7.2 ± 0.03	5.6	0.9	12.5
5f(*R,S*)	5-F	6.8 ± 0.02	8.2 ± 0.13	5.8	1	25
5g(*R,S*)	5-Cl	6.7 ± 0.04	8.1 ± 0.01	5.7	0.9	25
5h(*R,S*)	5-Br	6.8 ± 0.06	8.4 ± 0.07	5.7	0.8	40
5i(*R,S*)	6-Me	6.1 ± 0.01	7.8 ± 0.06	5.1	0.9	50
5j(*R,S*)	6-F	6.6 ± 0.05	8.4 ± 0.12	6.2	1	63
5k(*R*)	5-F,6-Cl	7.1 ± 0.02	8.0 ± 0.04	5.5	1	8
5l(*S*)	5-F,6-Cl	7.5 ± 0.04	8.9 ± 0.03	6.7	1	25
5m(*R*)	5-F,6-F	7.0 ± 0.03	8.4 ± 0.02	6.9	1	25
5n(*S*)	5-F,6-F	7.0 ± 0.02	9.0 ± 0.04	6.7	1	100
5o(*S*)	5-Cl,6-F	7.4 ± 0.02	8.7 ± 0.04	6.4	1	20
5p(*R*)	4-Cl,5-F	8.0 ± 0.03	8.9 ± 0.02	6.1	0.9	8
5q(*S*)	4-Cl,5-F	7.4 ± 0.03	8.9 ± 0.11	6.5	1	32
14a(*RS*)	5-OMe	5.5 ± 0.06	7.0 ± 0.01	inact		32
14b(*RS*)	6-OMe	6.4 ± 0.02	7.9 ± 0.04	5.7	1	32
14c(*RS*)	7-OMe	6.9 ± 0.06	9.0 ± 0.23	6.4	1	125
14d(*RS*)	8-OMe	6.4 ± 0.03	7.9 ± 0.08	5.2	0.7	32
14e(*R*)	7-OMe	6.9 ± 0.06	8.4 ± 0.04	5.1	0.8	32
14f(*S*)	7-OMe	6.9 ± 0.01	9.0 ± 0.2	6.6	1	125
14g(*S*)	7-F	6.7 ± 0.04	8.5 ± 0.05	6.2	1	63
14h(*S*)	7-Cl	6.7 ± 0.09	8.4 ± 0.02	5.6	0.8	50
14i(*S*)	7-Br	7.0 ± 0.03	8.4 ± 0.09	5.6	1	25
14j(*S*)	7-Me	7.2 ± 0.1	8.1 ± 0.08	6.3	1	8
14k(*S*)	4,4-Me, 7-Me	7.0 ± 0.04	8.5 ± 0.17	6.7	1	32
14l(*S*)	4,4-Me, 7-F	7.5 ±0.01	8.3 ± 0.09	6.7	1	6
14m(*S*)	4,4-Me, 7-OMe	8.0 ± 0.03	9.4 ± 0.1	7.0	1	25
15(*R,S*)		7.2 ± 0.02	8.2 ± 0.02	6.8	0.9	10

Table 2. ED_{50} (mg/kg) for Inducing Penile Erection in Rats after sc or po Administration for **5**, **14**, and **15**

5a	>10 sc	**14a**	>10 sc
5b	>10 sc	**14b**	>10 sc
5c	3.2 sc, >30 po	**14c**	0.6 sc, 11 po
5d	>10 sc	**14d**	>10 sc
5e	>10 sc	**14e**	2 sc, >30 po
5f	1 sc, 2.7 po	**14f**	1.2 sc, 10 po
5g	2.3 sc, >30 po	**14g**	1.5 sc, >30 po
5h	4 sc, >30 po	**14h**	2.7 sc, >30 po
5i	3.3 sc, >30 po	**14i**	>10 sc
5j	0.3 sc, >30 po	**14j**	>10 sc
5k	2.1 sc, >30 po	**14k**	0.9 sc, 10 po
5l	0.5 sc, 5.5 po	**14l**	2.7 sc, >30 po
5m	2.7 sc, 30 po	**14m**	0.5 sc, 10 po
5n	0.3 sc, 10 po	**15**	0.8 sc, 9.9 po
5o	1 sc, 15 po		
5p	3.3 sc, inact po		
5q	2.7 sc, >30 po		

Results

The radioligand binding experiments (Table 1) showed higher affinity of the indoles **5** and the indeno[1,2-*b*]-pyrroles **14** for $5HT_{2C}$ binding sites than for the structurally (79% homology between the transmembrane regions) very similar $5HT_{2A}$ receptor. Compounds with halogen substituents in position 4, 5, and 6 of the indole ring possess higher affinities for this receptor subtype as compared to derivatives bearing electron-donating substituents such as methoxy and methyl groups. The dihalogenated indoles showed the highest $5HT_{2C}$ receptor affinities. The (*S*)-enantiomers display higher af-

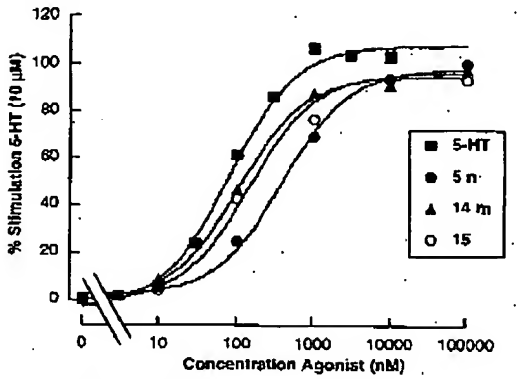
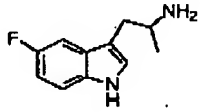


Figure 1. Effects of 5HT, **5n**, **14m**, and **15** on IP_3 formation in the rat choroid plexus. Results are expressed as percentage of the stimulation in IP_3 formation produced by 10 μM 5HT.

finity and selectivity for the $5HT_{2C}$ receptor as compared to their antipodes. Selectivity ratios of 20–100 were found for the (*S*)-configured isomers (e.g. **5l** and **5n**).

In the series of the indeno[1,2-*b*]pyrroles **14**, the optimal position for aromatic substitution turned out to be position 7. In contrast to the indoles, methoxy-substituted indeno[1,2-*b*]pyrroles (e.g. **14f** and **14m**) show increased affinities in comparison to the halogenated compounds and for the (*S*)-configured isomers selectivity ratios of up to 125 were observed.

For the study it was of interest to compare one of the potent and selective ligands with the structural isomer. The fluorinated tryptamine derivative **15** displays similar affinity for the $5HT_{2C}$ receptor as the isomer **5j**, but with reduced selectivity relative to the $5HT_{2A}$ receptor.



15

The effect of the $5HT_{2C}$ receptor ligands in stimulating phosphoinositol formation (cf. IP_3 formation, pEC_{50}, intrinsic activity, Table 1) was studied in rat choroid plexus. Compounds **5a–d**, **14a**, and **14d** induced only a submaximal increase whereas the maximum responses of the other derivatives **5e–q**, **14b**, **14c**, **14e–m**, and **15** were the same as that produced by 5HT (10^{-5} M, intrinsic activity = 1), suggesting that these ligands are full agonists at the $5HT_{2C}$ receptor (cf. **5n**, **14m**, and **15**, Figure 1). *In vivo* results, i.e. induction of penile erections, are presented in Table 2 (although not shown here, the reference compound fluoxetine was found to induce penile erection with ED_{50} = 4.3 mg/kg sc).

SSRIs such as fluoxetine are currently in use for the treatment of OCD. These drugs, however, exhibit a delayed onset of action and less than optimal therapeutic efficacy. Schedule-induced polydipsia in rats has been proposed as a model of OCD.[5] In this model, food-deprived rats which receive intermittently delivered food pellets on a fixed-time schedule typically develop a pattern of excessive drinking, i.e. polydipsia. This paradigm has been pharmacologically validated as a model of OCD. Experimental compounds are tested in this model for their ability to attenuate polydipsic behavior, i.e. for their potential anti-OCD effects. The

Table 3. Activity in Schedule-Induced Polydipsia Model in Rats

compd	min ED	max. suppression (%)	compd	min ED	max. suppression (%)
5f	3 ip	−97	**14c**	10 ip	−79
5l	10 ip	−90	**14f**	1 ip	−97
5n	3 ip	−78	**14k**	10 ip	−76
5o	3 ip	−88	**14m**	1 ip	−75
5q	3 ip	−97	fluoxetine	30 ip	−11
15	1 ip	−96			

selected $5HT_{2C}$ receptor agonists evaluated in the schedule-induced polydipsia model all significantly reduced the excessive drinking with MED values (minimal effective dose; i.e. the lowest dose tested which was found to statistically significantly reduce water intake relative to vehicle treatment) within the dose range 1–30 mg/kg (ip) with doses selected at half-logarithmic units (cf. Table 3). The magnitude of the suppression of polydipsia was compared among all of these compounds for the doses up to 10 mg/kg and was found to be 75% or more. In comparison, fluoxetine was much less potent, first achieving a statistically significant reduction in water intake of only 11% at 30 mg/kg ip (with no appreciable effect at doses up to 10 mg/kg ip).

Conclusions

Compounds were identified which exhibited high-affinity binding to human $5HT_{2C}$ receptors with selectivity versus $5HT_{2A}$ receptors. Such compounds were characterized *in vitro* and *in vivo* as $5HT_{2C}$ agonists. Two of these compounds underwent a broad binding evaluation: **5l** and **14k** exhibited affinity for several other 5HT receptor subtypes (1A, 3, 4, 6, 7) which was at least 2 logarithmic units lower than for $5HT_{2C}$ receptors and had $IC_{50} \geq 1$ μm for 26 other receptors across numerous different neurotransmitter systems (unpublished results). In the isolated rat fundus strip assay, both **5l** and **14k** act as agonists at the $5HT_{2B}$ receptor (pD_2 = 8.0 and 6.1, respectively; unpublished results). At present little is known concerning the physiological function of $5HT_{2B}$ receptors, due in part to the lack of highly selective ligands; interestingly a $5HT_{2C}$ receptor agonist which is structurally different from those described in this report and which exhibited antagonistic activity at the $5HT_{2B}$ receptor was also found to reduce schedule-induced polydipsia (unpublished results). Therefore the $5HT_{2B}$ receptor is unlikely to play a major role in the functional effects described here.

These $5HT_{2C}$ receptor agonists were found to significantly suppress schedule-induced polydipsia in rats, even at doses lacking any appreciable effects on spontaneous behavior. These results suggest that $5HT_{2C}$ receptor agonists may be of therapeutic value in OCD. In this respect, it is interesting to note that although fluoxetine was found to be active in this animal model of OCD, its potency was low when compared to the dose range producing adverse effects. $5HT_{2C}$ receptor agonists may, thus, potentially offer improved therapy of OCD.

Experimental Section

General. Melting points were determined in capillary tubes (Büchi 530 apparatus) and are uncorrected. Column chromatography was carried out by using silica gel (230–400

mesh; Merck) and 0.8–1.0 bar pressure. Spectra were recorded with the following instruments. IR (cm^{-1}): Nicolet-7199-FT-IR. ^{1}H-NMR (δ values in ppm relative to internal TMS, coupling constants J in Hertz): Bruker AC-250 (250 MHz). MS: MS9 updated with a Finnigan MAT data system SS 200. Elementary analyses (C, H, N) for novel compounds were within 0.4% of the theoretical values.

5-Methoxyindole, 4-methoxyindole, 4-methylindole, 5-methylindole, 5-fluoroindole, 5-chloroindole, 5-bromoindole, and 6-methylindole were purchased from Aldrich Chemicals; 4-fluoroindole and 6-fluoroindole, from Sigma; 5-methoxyindan-1-one and 6-methoxyindan-1-one, from Fluka.

Preparation of (*R*)-1-(6-Chloro-5-fluoroindol-1-yl)propan-2-ol (2l) (Standard Procedure A). To a mixture of sodium hydride (0.09 g, 3.7 mmol) in THF (15 mL) was added 6-chloro-5-fluoroindole (1k) (0.5 g, 3 mmol) at 0 °C. After 1 h (*R*)-propylene oxide (0.42 mL, 6 mmol) was added, and the mixture was stirred for 48 h at room temperature. The reaction was quenched with water, and the mixture was extracted with diethyl ether and washed with brine. The organic layer was dried, and the solvent was removed. The residue was subjected to chromatography (toluene/ethyl acetate, 19:1, as eluent) to yield 2l (0.51 g, 74%) as white crystals: mp 104–105 °C; $[\alpha]^{20}_{436} = -60.4°$ (c = 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.26 (d, J = 6 Hz, 3 H), 1.61 (d, J = 5 Hz, 1 H), 3.96 (dd, J = 12.5 Hz, J = 8 Hz, 1 H), 4.12 (dd, J = 12.5 Hz, J = 3.7 Hz, 1 H), 4.20 (m, 1 H), 6.45 (d, J = 3.2 Hz, 1 H), 7.17 (d, J = 3.2 Hz, 1 H), 7.34 (d, J = 9.5 Hz, 1 H), 7.38 (d, J = 7.5 Hz, 1 H); MS (EI) *m/z* 227 (M^+), 182 (100). Anal. ($C_{11}H_{11}ClFNO$) C, H, N.

Preparation of (*S*)-1-(2-Azidopropyl)-6-chloro-5-fluoroindole (4l) (Standard Procedure B). To a solution of 2l (0.28 g, 1.2 mmol) in dichloromethane (6 mL) and triethylamine (0.5 mL) was added methanesulfonyl chloride (0.2 mL, 2.5 mmol) at 0 °C. After 1 h ether was added and the mixture was extracted with 1 M sodium carbonate and washed with brine. The organic layer was dried, and the solvent was removed. The residue was taken up in DMF (6 mL), and sodium azide (0.16 g, 2.4 mmol) was added. The reaction mixture was heated for 7 h at 60 °C, poured into water, and extracted with ether. The organic layer was dried, and the solvent was removed. The residue was purified by column chromatography (toluene as eluent) to yield 4l as yellow oil (0.29 g, 93%): ^{1}H NMR ($CDCl_3$) δ 1.30 (d, J = 6.2 Hz, 3 H), 3.90 (m, 1 H), 4.00 (dd, J = 15, 7 Hz, 1 H), 4.07 (dd, J = 15, 5 Hz, 1 H), 6.48 (d, J = 2.5 Hz, 1 H), 7.14 (d, J = 2.5 Hz, 1 H), 7.34 (d, J = 5 Hz, 1 H), 7.35 (d, J = 10 Hz, 1 H); MS (EI) *m/z* 252 (M^+), 182 (100).

Preparation of (*S*)-2-(6-Chloro-5-fluoroindol-1-yl)-1-methylethylamine Fumarate (1:1.6) (5l) (Standard Procedure C). A suspension of 0.02 g of PtO_2 in ethanol (5 mL) was stirred under hydrogen for 0.5 h. After the addition of a solution of 4l (0.26 g, 1 mmol) in ethanol (5 mL), the mixture was stirred for 2 h, the catalyst was filtered off, and the solvent was removed. The salt was prepared in ether by treatment with fumaric acid to yield 0.25 g (59%) of **5l**: mp 169–171 °C; $[\alpha]^{20}_{436} = 31.6°$ (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.10 (d, J = 5 Hz, 3 H), 3.51 (m, 1 H), 4.18 (dd, J = 14.5, 7.2 Hz, 1 H), 4.36 (dd, J = 14.5, 6.2 Hz, 1 H), 6.48 (d, J = 2.5 Hz, 1 H), 6.49 (s, 2 H), 7.40 (d, J = 2.5 Hz, 1 H), 7.35 (s, 1 H), 7.42 (d, J = 8 Hz, 1 H); MS (EI) *m/z* 226 (M^+), 183, 44 (100). Anal. ($C_{11}H_{12}FClN_2 \cdot 1.6C_4H_4O_4$) C, H, N.

Compounds **5a–k** and **5m–q** were synthesized according to standard procedures A, B, and C.

***(R,S)*-2-(5-Methoxyindol-1-yl)-1-methylethylamine fumarate (1:1) (5a):** 62%; mp 175–176 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.07 (d, J = 6.5 Hz, 3 H), 3.50 (sept, J = 6.5 Hz, 1 H), 3.74 (s, 3 H), 4.16 (dd, J = 15.3, 7.5 Hz, 1 H), 4.36 (dd, J = 15.3, 5.7 Hz, 1 H), 6.37 (d, J = 3 Hz, 1 H), 6.49 (s, 2 H), 6.78 (dd, J = 10, 2.5 Hz, 1 H), 7.05 (d, J = 2.5 Hz, 1 H), 7.33 (d, J = 3 Hz, 1 H), 7.45 (d, J = 10 Hz, 1 H); MS (EI) *m/z* 204 (M^+), 161, 44 (100). Anal. ($C_{12}H_{16}N_2O \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(4-Methoxyindol-1-yl)-1-methylethylamine fumarate (1:0.5) (5b):** 59%; mp 185–186 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.00 (d, J = 6.5 Hz, 3 H), 3.36 (sept, J = 6.5 Hz, 1 H), 3.86 (s, 1 H), 4.07 (dd, J = 15.3, 7 Hz, 1 H), 4.22 (dd, J = 15.3, 5 Hz, 1 H), 6.43 (d, J = 3 Hz, 1 H), 6.44 (s, 1 H), 6.52 (d, J = 7.5 Hz, 1 H), 7.05 (t, J = 7.5 Hz, 1 H), 7.13 (d, J = 7.5 Hz, 1 H), 7.25 (d, J = 3 Hz, 1 H); MS (EI) *m/z* 204 (M^+), 161, 44 (100). Anal. ($C_{12}H_{16}N_2O \cdot 0.5C_4H_4O_4$) C, H, N.

***(R,S)*-2-(4-Methylindol-1-yl)-1-methylethylamine fumarate (1:1) (5c):** 92%; mp 163–164 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.08 (d, J = 6.5 Hz, 3 H), 2.46 (s, 3 H), 3.52 (m, 1 H), 4.19 (dd, J = 14.2, 7.5 Hz, 1 H), 4.38 (dd, J = 14.2, 8.2 Hz, 1 H), 6.49 (s, 3 H), 6.83 (d, J = 7 Hz, 1 H), 7.04 (dd, J = 7.5, 7 Hz, 1 H), 7.37 (d, J = 3.2 Hz, 1 H), 7.37 (d, J = 7.5 Hz, 1 H); MS (EI) *m/z* 188 (M^+), 145, 44 (100). Anal. ($C_{12}H_{16}N_2 \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(4-Fluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5d):** mp 179–180 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.09 (d, J = 7.5 Hz, 3 H), 3.53 (m, 1 H), 4.24 (dd, J = 15, 7.2 Hz, 1 H), 4.42 (dd, J = 15, 7.5 Hz, 1 H), 6.49 (s, 2 H), 6.54 (d, J = 3 Hz, 1 H), 6.82 (dd, J = 8, 7.7 Hz, 1 H), 7.13 (m, 1 H), 7.43 (d, J = 9 Hz, 1 H), 7.46 (d, J = 3 Hz, 1 H); MS (EI) *m/z* 192 (M^+), 149, 44 (100). Anal. ($C_{11}H_{13}FN_2 \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(5-Methylindol-1-yl)-1-methylethylamine fumarate (1:1) (5e):** 87%; mp 165–167 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.06 (d, J = 6.5 Hz, 3 H), 2.36 (s, 3 H), 3.50 (m, 1 H), 4.18 (dd, J = 14.2, 7.5 Hz, 1 H), 4.38 (dd, J = 14.2, 5.7 Hz, 1 H), 6.36 (d, J = 3 Hz, 1 H), 6.49 (s, 3 H), 6.97 (d, J = 7 Hz, 1 H), 7.33 (dd, J = 7.5, 7 Hz, 1 H), 7.43 (d, J = 3.2 Hz, 1 H); MS (EI) *m/z* 188 (M^+), 145, 44 (100). Anal. ($C_{12}H_{16}N_2 \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(5-Fluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5f):** 95%; mp 169–170 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.08 (d, J = 6.5 Hz, 3 H), 3.52 (m, 1 H), 4.22 (dd, J = 14.5, 7.5 Hz, 1 H), 4.39 (dd, J = 14.5, 6 Hz, 1 H), 6.46 (d, J = 3 Hz, 1 H), 6.49 (s, 2 H), 7.00 (dt, J = 7.5, 2.5 Hz, 1 H), 7.32 (dd, J = 10, 2.5 Hz, 1 H), 7.47 (d, J = 3 Hz, 1 H), 7.58 (dd, J = 9, 4.5 Hz, 1 H); MS (EI) *m/z* 192 (M^+), 149, 44 (100). Anal. ($C_{11}H_{13}FN_2 \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(5-Chloroindol-1-yl)-1-methylethylamine fumarate (1:2) (5g):** mp 183–185 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.11 (d, J = 6.5 Hz, 3 H), 3.57 (m, 1 H), 4.25 (dd, J = 14.2, 7.5 Hz, 1 H), 4.42 (dd, J = 14.2, 6.2 Hz, 1 H), 6.48 (d, J = 3 Hz, 1 H), 6.54 (s, 2 H), 7.16 (dd, J = 7.5, 2.5 Hz, 1 H), 7.48 (d, J = 3 Hz, 1 H), 7.61 (d, J = 7.5 Hz, 1 H), 7.61 (d, J = 2.5 Hz, 1 H); MS (EI) *m/z* 208 (M^+), 165, 44 (100). Anal. ($C_{11}H_{13}ClN_2 \cdot 2C_4H_4O_4$) C, H, N.

***(R,S)*-2-(5-Bromoindol-1-yl)-1-methylethylamine fumarate (1:1) (5h):** 93%; mp 196–197 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.05 (d, J = 6.5 Hz, 3 H), 3.49 (m, 1 H), 4.20 (dd, J = 14.5, 7 Hz, 1 H), 4.36 (dd, J = 14.5, 6.2 Hz, 1 H), 6.46 (d, J = 3.2 Hz, 1 H), 6.49 (s, 2 H), 7.26 (dd, J = 8.7, 2 Hz, 1 H), 7.45 (d, J = 3.2 Hz, 1 H), 7.55 (d, J = 8.7 Hz, 1 H), 7.74 (d, J = 2 Hz, 1 H); MS (EI) *m/z* 252, 254 (M^+), 211, 209, 44 (100). Anal. ($C_{11}H_{13}BrN_2 \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(6-Methylindol-1-yl)-1-methylethylamine fumarate (1:1) (5i):** 60%; mp 152–153 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.09 (d, J = 6.5 Hz, 3 H), 2.41 (s, 3 H), 3.53 (m, 1 H), 4.16 (dd, J = 14.5, 7.5 Hz, 1 H), 4.36 (dd, J = 14.5, 5.7 Hz, 1 H), 6.39 (d, J = 3 Hz, 1 H), 6.50 (s, 2 H), 6.87 (d, J = 8 Hz, 1 H), 7.29 (d, J = 3 Hz, 1 H), 7.35 (s, 1 H), 7.42 (d, J = 8 Hz, 1 H); MS (EI) *m/z* 188 (M^+), 145, 44 (100). Anal. ($C_{12}H_{16}N_2 \cdot C_4H_4O_4$) C, H, N.

***(R,S)*-2-(6-Fluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5j):** 78%; mp 158–159 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.10 (d, J = 6.5 Hz, 3 H), 3.51 (m, 1 H), 4.18 (dd, J = 14.5, 7.2 Hz, 1 H), 4.36 (dd, J = 14.5, 6.2 Hz, 1 H), 6.48 (d, J = 2.5 Hz, 1 H), 6.49 (s, 2 H), 6.89 (dt, J = 8.7, 2.2 Hz, 1 H), 7.40 (d, J = 2.5 Hz, 1 H), 7.47 (dd, J = 10.5, 2.2 Hz, 1 H), 7.54 (dd, J = 8.7, 5.5 Hz, 1 H); MS (EI) *m/z* 192 (M^+), 149, 44 (100). Anal. ($C_{11}H_{13}FN_2 \cdot C_4H_4O_4$) C, H, N.

***(R)*-2-(6-Chloro-5-fluoroindol-1-yl)-1-methylethylamine fumarate (1:1.5) (5k):** 69%; mp 153–154 °C dec; $[\alpha]^{20}_{436} = -28.8°$ (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.10 (d, J = 5 Hz, 3 H), 3.51 (m, 1 H), 4.18 (dd, J = 14.5, 7.2 Hz, 1 H), 4.36 (dd, J = 14.5, 6.2 Hz, 1 H), 6.48 (d, J = 2.5 Hz, 1 H), 6.49 (s, 2 H), 7.40 (d, J = 2.5 Hz, 1 H), 7.35 (s, 1 H), 7.42 (d, J = 8 Hz, 1 H). Anal. ($C_{11}H_{12}FClN_2 \cdot 1.5C_4H_4O_4$) C, H, N.

(*R*)-2-(5,6-Difluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5m): 82%; mp 161−162 °C dec; $[\alpha]^{20}_{436} = -34.4°$ (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.09 (d, J = 6.5 Hz, 3 H), 3.49 (m, 1 H), 4.19 (dd, J = 14.5, 7 Hz, 1 H), 4.33 (dd, J = 14.5, 6.2 Hz, 1 H), 6.48 (d, J = 3.2 Hz, 1 H), 6.49 (s, 2 H), 7.47 (d, J = 3.2 Hz, 1 H), 7.54 (dd, J = 11.2, 7.5 Hz, 1 H), 7.74 (dd, J = 11.7, 7 Hz, 1 H); MS (EI) m/z 210 (M^+), 167, 166, 44 (100). Anal. ($C_{11}H_{12}F_2N_2 \cdot C_4H_4O_4$) C, H, N.

(*S*)-2-(5,6-Difluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5n): 84%; mp 159−160 °C dec; $[\alpha]^{20}_{436} = +35.2°$ (c = 0.25, MeOH). Anal. ($C_{11}H_{12}F_2N_2 \cdot C_4H_4O_4$) C, H, N.

(*S*)-2-(5-Chloro-6-fluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5o): mp 158−160 °C dec; $[\alpha]^{20}_D = +35.2°$ (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.09 (d, J = 6.7 Hz, 3 H), 3.50 (m, 1 H), 4.20 (dd, J = 14.5, 7.5 Hz, 1 H), 4.34 (dd, J = 14.5, 6.5 Hz, 1 H), 6.49 (s, 2 H), 6.49 (d, J = 3.2 Hz, 1 H), 7.48 (d, J = 3.2 Hz, 1 H), 7.73 (d, J = 7.5 Hz, 1 H), 7.74 (d, J = 10 Hz, 1 H); MS (EI) m/z 226 (M^-), 183, 44 (100). Anal. ($C_{11}H_{12}FClN_2 \cdot C_4H_4O_4$) C, H, N.

(*R*)-2-(4-Chloro-5-fluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5p): 84%; mp 186−187 °C dec; $[\alpha]^{20}_{436} = -32.4°$ (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.08 (d, J = 6.5 Hz, 3 H), 3.48 (m, 1 H), 4.23 (dd, J = 14.5, 7 Hz, 1 H), 4.36 (dd, J = 14.5, 6.5 Hz, 1 H), 6.49 (s, 2 H), 6.54 (d, J = 3.2 Hz, 1 H), 7.19 (t, J = 10 Hz, 1H), 7.60 (d, J = 3.2 Hz, 1 H), 7.42 (d, J = 8 Hz, 1 H); MS (EI) m/z 226 (M^+), 183, 44 (100). Anal. ($C_{11}H_{12}FClN_2 \cdot C_4H_4O_4$) C, H, N.

(*S*)-2-(4-Chloro-5-fluoroindol-1-yl)-1-methylethylamine fumarate (1:1) (5q): 84%; mp 183−184 °C dec; $[\alpha]^{20}_{436} = +32.4°$ (c = 0.25, MeOH). Anal. ($C_{11}H_{12}FClN_2 \cdot C_4H_4O_4$) C, H, N.

***N*-(2-Bromo-5-chloro-4-fluorophenyl)trifluoroacetamide (7).** To a solution of 2-bromo-4-chloro-3-fluorophenylamine (6) (111 g, 0.5 mol) in ether (990 mL) at 0 °C were added solid Na_2CO_3 (78 g) and trifluoroacetic anhydride (86 mL, 0.9 mol). After being warmed to room temperature the suspension was stirred for 2.5 h, diluted with ether, and extracted with water. The organic layer was separated and dried (sodium sulfate), and the solvent was removed. The residue was recrystallized from *n*-hexane to yield 7 as yellow crystals (136 g, 86%): mp 86−88 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (d, J = 7.6 Hz, 1 H), 8.46 (d, J = 7 Hz, 1 H); MS (EI) m/z 319, 321 (M^+), 240 (100). Anal. ($C_8H_3F_4ClBrNO$) C, H, N.

(*R,S*)-1-(Trifluoroacetyl)-6-chloro-5-fluoro-3-hydroxy-2,3-dihydro-1*H*-indole (8). A solution of 7 (24 g, 75 mmol) in THF (750 mL) was cooled to −100 °C. MeLi (75 mmol, 1.6 M in ether) was added, and 10 min later *t*-BuLi (150 mL, 1.7 M in pentane) was also added. After 1 h at −100 °C a solution of chloroacetaldehyde (67.5 mL, 1.7 M in THF) was added. The mixture was stirred at −78 °C for 4 h, and acetic acid (13 mL) was added. After the addition of triethylamine (52 mL), the reaction mixture was allowed to warm to ambient temperature and to stir for 14 h. Ammonium chloride solution (20%, 150 mL) was added, followed by extraction with ether. The organic layer was separated and dried (sodium sulfate), and the solvent was removed. The residue was purified by column chromatography (toluene/ethyl acetate, 19:1) to yield 8 (13g, 61%): mp 97.5−98 °C; ^{1}H NMR ($CDCl_3$) δ 2.36 (d, J = 7 Hz, 1 H), 5.37 (m, 1 H), 7.26 (d, J = 7.7 Hz, 1 H), 8.35 (d, J = 6.25 Hz, 1 H); MS (EI) m/z 283 (M^+), 186, 69 (100). Anal. ($C_{10}H_6F_4ClNO_2$) C, H, N.

6-Chloro-5-fluoroindole (1k). A solution of 8 (5.5 g, 19.5 mmol) and *p*-toluenesulfonic acid monohydrate (0.19 g, 0.9 mmol) in toluene (200 mL) was heated at reflux temperature for 2 h. The solvent was removed, and the residue was dissolved in methanol (800 mL). After the addition of NaOH (1 N, 800 mL), the mixture was refluxed for 2.5 h. Methanol was removed, and the crystals were filtered off and dried: yield 77%; mp 104−105 °C; ^{1}H NMR ($CDCl_3$) δ 6.50 (m, 1 H), 7.24 (m, 1 H), 7.36 (d, J = 9.5 Hz, 1 H), 7.41 (d, J = 6 Hz, 1 H); MS (EI) m/z 169 (M^-), 134, 107 (100).

Preparation of (*R,S*)-2-(2-Buten-1-yl)-3,3,6-trimethyl-1-indanone (10i) (Standard Procedure D). A solution of 3,3,6-trimethylindan-1-one (9i)[12−14] (18.9 g, 108 mmol), 3-buten-2-ol (22.4 mL, 0.26 mol), and *p*-toluenesulfonic acid (300 mg) in 2,2-dimethoxypropane (200 mL) was boiled under reflux for 64 h on a Dean−Stark trap filled with molecular sieves (0.4 nm, 2 mm pearl shaped). The solvents were evaporated, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 6:1) to give **10i** (12.7 g, 51%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 3 H), 1.45 (s, 3 H), 1.69 (d, J = 2 Hz, 3 H), 2.18 (m, 1 H), 2.39 (s, 3 H), 2.43 (m, 1 H), 2.69 (m, 1 H), 5.58 (m, 2 H), 7.41 (m, 2 H), 7.50 (s, 1 H); MS (EI) m/z 228 (M^+), 213 (M^+ − Me), 173 (100), 159, 115, 55.

Compounds **10a−b** and **10j−k** were prepared in the same way from 4-methoxyindan-1-one (**9a**),[15] 5-methoxyindan-1-one (**9b**), 6-methoxyindan-1-one (**9c**), 7-methoxyindan-1-one (**9d**),[16] 6-fluoroindan-1-one (**9e**),[17] 6-chloroindan-1-one (**9f**),[16] 6-bromoindan-1-one (**9g**),[17,18] 6-methylindan-1-one (**9h**),[19] 6-fluoro-3,3-dimethylindan-1-one (**9j**),[12−14,17] and 6-methoxy-3,3-dimethylindan-1-one (**9k**),[12−15] respectively.

Preparation of (*R,S*)-2-(2-Oxoethyl)-3,3,6-trimethyl-1-indanone (11i) (Standard Procedure E). An ozone stream (2.5 g of ozone/h) was conducted for 1 h while stirring through a solution of **10i** (12.7 g, 55.6 mmol) in dichloromethane (200 mL) and methanol (40 mL) cooled to −70 °C. Subsequently, the solution was flushed with oxygen for 5 min and with argon for 10 min. After the addition of dimethyl sulfide (6.12 mL, 83.4 mmol), the mixture was stirred at room temperature for 18 h. The reaction mixture was evaporated, the residue was treated with dichloromethane (150 mL) and after the addition of water (25 mL) and trifluoroacetic acid (25 mL), the mixture was stirred at room temperature for 2.5 h. The mixture was subsequently poured into water (150 mL) and neutralized while stirring by addition of hydrogen carbonate. Water (100 mL) was added, the phases were separated, and the aqueous phase was extracted twice with dichloromethane (150 mL each time). The combined organic phases were dried (magnesium sulfate) and concentrated to give **11i** (11.3 g, 94%) as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 3 H), 1.51 (s, 3 H), 2.41 (s, 3 H), 2.61 (m, 1 H), 3.04 (m, 2 H), 7.40 (d, J = 7 Hz, 1 H), 7.46 (d, J = 7 Hz, 1 H), 7.52 (s, 1 H), 9.99 (s, 1 H); MS (EI) m/z 188 (M^+ − CO), 173 (100), 159, 145, 128, 43.

Preparation of (*R*)-1-(4,4,7-Trimethyl-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)propan-2-ol (12k) (Standard Procedure F). A solution of **11i** (2.16 g, 10 mmol) and *p*-toluenesulfonic acid (80 mg) in toluene (90 mL) was heated on a Dean−Stark trap. A solution of (*R*)-1-amino-2-propanol (3.0 g, 40 mmol) in toluene (20 mL) was added dropwise over a period of 5 min. Subsequently, the mixture was boiled for an additional 45 min, during which the solvent was reduced to a volume of 20 mL. The cooled reaction mixture was purified by column chromatography (ethyl acetate/hexane, 1:2) to yield **12k** (1.5 g, 59%) as a brown oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (d, J = 5 Hz, 3 H), 1.41 (s, 6 H), 2.38 (s, 3 H), 3.99 (m, 1 H), 4.18 (m, 2 H), 6.11 (d, J = 2 Hz, 1 H), 6.68 (d, J = 2 Hz, 1 H), 6.90 (d, J = 7 Hz, 1 H), 7.07 (s, 1 H), 7.21 (d, J = 7 Hz, 1 H); MS (EI) m/z 255 (M^+), 240 (100), 194.

Preparation of (*S*)-1-(2-Azidopropyl)-4,4,7-trimethyl-1,4-dihydroindeno[1,2-*b*]pyrrole (13k) (Standard Procedure G). Methanesulfonyl chloride (0.91 mL, 11.7 mmol) was added dropwise while stirring to a solution, cooled to 0 °C, of **12k** (1.5 g, 5.87 mmol) and triethylamine (3.27 mL, 23.5 mmol) in dichloromethane (50 mL), and the mixture was stirred at this temperature for an additional 1.5 h. The reaction mixture was subsequently diluted with dichloromethane (150 mL), washed twice with saturated sodium hydrogen carbonate solution (70 mL each time) and once with brine (70 mL), dried (magnesium sulfate), and evaporated. The residue was dissolved in DMF (50 mL) and treated with sodium azide (0.76 g, 11.7 mmol) and the mixture was heated to 60 °C for 15 h while stirring. After cooling the solution was poured into water (100 mL) and extracted twice with ethyl acetate (100 mL each time). The combined organic phases were washed once with water (100 mL) and once with brine (100 mL), dried (magnesium sulfate) and evaporated. The residue was purified by column chromatography (hexane/ethyl acetate 4:1) to give **13k** (1.13 g, 68%) as a reddish oil: ^{1}H NMR ($CDCl_3$) δ 1.30 (d, J = 5 Hz, 3 H), 1.41 (s, 6 H), 2.39 (s, 3 H), 3.92 (m, 1 H), 4.09 (m, 2 H), 6.12 (d, J = 2 Hz, 1 H), 6.65 (d, J = 2 Hz, 1 H), 6.90

(d, J = 7 Hz, 1 H), 7.03 (s, 1 H), 7.21 (d, J = 7 Hz, 1 H); MS (EI) *m/z* 280 (M^+), 237 (100), 194, 181, 56.

Preparation of (*S*)-2-(4,4,7-Trimethyl-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine Fumarate (1:1) (14k) (Standard Procedure H). **13k** (1.1 g, 3.92 mmol) dissolved in ethanol (50 mL) was hydrogenated over platinum oxide (110 mg) for 4 h at room temperature. The catalyst was subsequently filtered off and rinsed with ethanol, and the solution was evaporated. The colorless residue was dissolved in ether (80 mL), filtered, and treated while stirring with a solution of fumaric acid (455 mg, 3.92 mmol) in methanol (15 mL). The mixture was stirred at room temperature for 24 h, and the crystals were subsequently filtered off to give **14k** (805 mg, 77%) as a white solid: mp 196 °C; $[\alpha]^{20}_D$ = +11.2° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.08 (d, J = 5 Hz, 3 H), 1.32 (s, 6 H), 2.32 (s, 3 H), 3.44 (m, 1 H), 4.14 (dd, J = 10, 7 Hz, 1 H), 4.41 (dd, J = 10, 4 Hz, 1 H), 6.06 (d, J = 2 Hz, 1 H), 6.51 (s, 2 H), 6.79 (d, J = 2 Hz, 1 H), 6.84 (d, J = 7 Hz, 1 H), 7.23 (d, J = 7 Hz, 1 H), 7.33 (s, 1 H); MS (EI) *m/z* 254 (M^+), 211, 196, 44 (100). Anal. ($C_{17}H_{22}N_2 \cdot 1C_4H_4O_4$) C, H, N.

Compounds **14a–j** and **14l,m** were synthesized according to standard procedures D, E, F, G, and H.

(*R,S*)-2-(5-Methoxy-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14a): 83%; mp 194 °C; ^{1}H NMR (DMSO-d_6) δ 1.01 (d, J = 5 Hz, 3 H), 3.30 (s, 3 H), 3.31 (m, 1 H), 3.83 (s, 3 H), 4.08 (dd, J = 10, 7 Hz, 1 H), 4.25 (dd, J = 10, 4 Hz, 1 H), 6.11 (d, J = 2 Hz, 1 H), 6.45 (s, 1 H), 6.76 (d, J = 7 Hz, 1 H), 6.85 (d, J = 2 Hz, 1 H), 7.22 (m, 2 H); MS (EI) *m/z* 242 (M^+), 199, 44 (100). Anal. ($C_{15}H_{18}N_2O \cdot 0.5C_4H_4O_4 \cdot 0.12$MeOH) C, H, N.

(*R,S*)-2-(6-Methoxy-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.6) (14b): 61%; mp 189 °C; ^{1}H NMR (DMSO-d_6) δ 1.02 (d, J = 5 Hz, 3 H), 3.33 (m, 1 H), 3.39 (s, 3 H), 3.75 (s, 3 H), 4.05 (dd, J = 10, 7 Hz, 1 H), 4.23 (dd, J = 10, 4 Hz, 1 H), 6.08 (d, J = 2 Hz, 1 H), 6.46 (s, 1.2 H), 6.77 (d, J = 2 Hz, 1 H), 6.79 (dd, J = 7, 2 Hz, 1 H), 7.08 (d, J = 2 Hz, 1 H), 7.44 (d, J = 7 Hz, 1 H); MS (EI) *m/z* 242 (M^+), 199, 44 (100). Anal. ($C_{15}H_{18}N_2O \cdot 0.6C_4H_4O_4$) C, H, N.

(*R,S*)-2-(7-Methoxy-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14c): 79%; mp 203 °C; ^{1}H NMR (DMSO-d_6) δ 1.01 (d, J = 5 Hz, 3 H), 3.33 (m, 1 H), 3.34 (s, 3 H), 3.78 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.11 (d, J = 2 Hz, 1 H), 6.44 (s, 1 H), 6.63 (dd, J = 7, 2 Hz, 1 H), 6.85 (d, J = 2 Hz, 1 H), 7.08 (d, J = 2 Hz, 1 H), 7.29 (d, J = 7 Hz, 1 H); MS (EI) *m/z* 242 (M^+), 199, 44 (100). Anal. ($C_{15}H_{18}N_2O \cdot 0.5C_4H_4O_4$) C, H, N.

(*R,S*)-2-(8-Methoxy-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.52) (14d): 74%; mp 193 °C; ^{1}H NMR (DMSO-d_6) δ 1.01 (d, J = 5 Hz, 3 H), 3.32 (m, 1 H), 3.41 (s, 3 H), 3.92 (s, 3 H), 4.21 (dd, J = 10, 7 Hz, 1 H), 4.38 (dd, J = 10, 4 Hz, 1 H), 6.12 (d, J = 2 Hz, 1 H), 6.43 (s, 1.04 H), 6.84 (d, J = 2 Hz, 1 H), 6.96 (m, 1 H), 7.05 (m, 2 H); MS (EI) *m/z* 242 (M^+), 199, 44 (100). Anal. ($C_{16}H_{18}N_2O \cdot 0.52C_4H_4O_4$) C, H, N.

(*R*)-2-(7-Methoxy-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14e): 68%; mp 207 °C; $[\alpha]^{20}_D$ = −21.6° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.02 (d, J = 5 Hz, 3 H), 3.32 (m, 1 H), 3.34 (s, 3 H), 3.78 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.10 (d, J = 2 Hz, 1 H), 6.45 (s, 1 H), 6.61 (dd, J = 7, 2 Hz, 1 H), 6.86 (d, J = 2 Hz, 1 H), 7.09 (d, J = 2 Hz, 1 H), 7.29 (d, J = 7 Hz, 1 H); MS (EI) *m/z* 242 (M^+), 199, 44 (100). Anal. ($C_{15}H_{18}N_2O \cdot 0.5C_4H_4O_4$) C, H, N.

(*S*)-2-(7-Methoxy-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14f): 77%; mp 206 °C; $[\alpha]^{20}_D$ = +23.2° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.01 (d, J = 5 Hz, 3 H), 3.32 (m, 1 H), 3.34 (s, 3 H), 3.78 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.10 (d, J = 2 Hz, 1 H), 6.44 (s, 1 H), 6.62 (dd, J = 7, 2 Hz, 1 H), 6.86 (d, J = 2 Hz, 1 H), 7.09 (d, J = 2 Hz, 1 H), 7.29 (d, J = 7 Hz, 1 H); MS (EI) *m/z* 242 (M^+), 199, 44 (100). Anal. ($C_{15}H_{18}N_2O \cdot 0.5C_4H_4O_4$) C, H, N.

(*S*)-2-(7-Fluoro-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14g): 54%; mp 194 °C; $[\alpha]^{20}_D$ = +16.8° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.02 (d, J = 5 Hz, 3 H), 3.29 (m, 1 H), 3.40 (s, 3 H), 4.09 (dd, J = 10, 7 Hz, 1 H), 4.23 (dd, J = 10, 4 Hz, 1 H), 6.14 (d, J = 2 Hz, 1 H), 6.45 (s, 1 H), 6.83 (dt, J = 7, 1 Hz, 1 H), 6.91 (d, J = 2 Hz, 1 H), 7.40 (m, 2 H); MS (EI) *m/z* 230 (M^+), 187, 44 (100). Anal. ($C_{14}H_{16}FN_2 \cdot 0.5C_4H_4O_4$) C, H, F, N.

(*S*)-2-(7-Chloro-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.55) (14h): 67%; mp 197 °C; $[\alpha]^{20}_D$ = +16.0° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.01 (d, J = 5 Hz, 3 H), 3.30 (m, 1 H), 3.43 (s, 3 H), 4.08 (dd, J = 10, 7 Hz, 1 H), 4.27 (dd, J = 10, 4 Hz, 1 H), 6.15 (d, J = 2 Hz, 1 H), 6.46 (s, 1.1 H), 6.93 (d, J = 2 Hz, 1 H), 7.07 (dd, J = 7, 1 Hz, 1 H), 7.41 (d, J = 7 Hz, 1 H), 7.60 (d, J = 1 Hz, 1 H); MS (EI) *m/z* 246 (M^+), 203, 44 (100). Anal. ($C_{14}H_{15}ClN_2 \cdot 0.55C_4H_4O_4$) C, H, Cl, N.

(*S*)-2-(7-Bromo-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14i): 50%; mp 197 °C; $[\alpha]^{20}_D$ = +14.8° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.01 (d, J = 5 Hz, 3 H), 3.29 (m, 1 H), 3.41 (s, 3 H), 4.09 (dd, J = 10, 7 Hz, 1 H), 4.26 (dd, J = 10, 4 Hz, 1 H), 6.15 (d, J = 2 Hz, 1 H), 6.46 (s, 1 H), 6.93 (d, J = 2 Hz, 1 H), 7.21 (dd, J = 7, 1 Hz, 1 H), 7.35 (d, J = 7 Hz, 1 H), 7.71 (d, J = 1 Hz, 1 H); MS (EI) *m/z* 290,292 (M^+), 247,249, 44 (100). Anal. ($C_{14}H_{15}BrN_2O \cdot 0.5C_4H_4O_4$) C, H, Br, N.

(*S*)-2-(7-Methyl-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:0.5) (14j): 65%; mp 194 °C; $[\alpha]^{20}_D$ = +22.5° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.03 (d, J = 5 Hz, 3 H), 2.35 (s, 3 H), 3.34 (m, 1 H), 3.36 (s, 3 H), 4.07 (dd, J = 10, 7 Hz, 1 H), 4.28 (dd, J = 10, 4 Hz, 1 H), 6.10 (d, J = 2 Hz, 1 H), 6.46 (s, 1 H), 6.85 (m, 2 H), 7.28 (d, J = 7 Hz, 1 H), 7.38 (s, 1 H); MS (EI) *m/z* 226 (M^+), 183, 44 (100). Anal. ($C_{15}H_{18}N_2 \cdot 0.5C_4H_4O_4$) C, H, N.

(*S*)-2-(7-Fluoro-4,4-dimethyl-1,4-dihydroindeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:1) (14l): 70%; mp 211 °C; $[\alpha]^{20}_D$ = +8.8° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.05 (d, J = 5 Hz, 3 H), 1.35 (s, 6 H), 3.41 (m, 1 H), 4.11 (dd, J = 10, 7 Hz, 1 H), 4.36 (dd, J = 10, 4 Hz, 1 H), 6.09 (d, J = 2 Hz, 1 H), 6.49 (s, 2 H), 6.83 (m, 1 H), 6.87 (d, J = 2 Hz, 1 H), 7.36 (m, 2 H); MS (EI) *m/z* 258 (M^+), 215, 200, 44 (100). Anal. ($C_{16}H_{19}FN_2 \cdot 1C_4H_4O_4$) C, H, F, N.

(*S*)-2-(7-methoxy-4,4-dimethyl-1,4-dihydro-indeno[1,2-*b*]pyrrol-1-yl)-1-methylethylamine fumarate (1:1) (14m): 60%; mp 181 °C; $[\alpha]^{20}_D$ = +10.0° (c = 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.05 (d, J = 5 Hz, 3 H), 1.32 (s, 6 H), 3.41 (m, 1 H), 3.77 (s, 3 H), 4.09 (dd, J = 10, 7 Hz, 1 H), 4.33 (dd, J = 10, 4 Hz, 1 H), 6.06 (d, J = 2 Hz, 1 H), 6.47 (s, 2 H), 6.59 (dd, J = 7, 1.5 Hz, 1 H), 6.80 (d, J = 2 Hz, 1 H), 7.08 (d, J = 1.5 Hz, 1 H), 7.24 (d, J = 7 Hz, 1 H); MS (EI) *m/z* 270 (M^+), 227, 212, 44 (100). Anal. ($C_{17}H_{22}N_2O \cdot 1C_4H_4O_4$) C, H, N.

Cell Culture and Membrane Preparation. Membranes obtained from NIH 3T3 cell lines expressing either human $5HT_{2A}$ or human $5HT_{2C}$ receptors were kindly donated by Dr. Nico Stam (N. V. Organon). For each receptor subtype, a single batch of membranes were grown using fermentation techniques previously described.[20]

Radioligand Binding Assays. Radioligand binding assays were as previously described for the human $5HT_{2A}$ receptor with minor modifications for the labeling of human $5HT_{2C}$ receptors. Briefly, on the day of the experiment, membranes were thawed and resuspended in 10 times the original volume of assay buffer. This gives a concentration of approximately 4×10^5 cells per assay tube. This assay buffer consisted of Tris-HCl 50 mM, pargyline 10^{-5} M, $MgCl_2$ 5 mM and ascorbic acid 0.1% pH 7.4. All compounds were dissolved in 10% DMSO and diluted in assay buffer. Assays were similar for each receptor and consisted of 100 μL of membrane preparation (depending on the assay), 50 μL of radioligand ([^{3}H]-5HT 1 nM final concentration for labeling human $5HT_{2C}$ receptor binding sites, and [^{3}H]DOB 1 nM final concentration for labeling human $5HT_{2A}$ receptors). Nonspecific binding was defined in the presence of 10 μM 5HT in the case of the human $5HT_{2C}$ receptor and 10 μM methysergide in the case of the human $5HT_{2A}$ receptor. All incubations were performed at room temperature for 1 h and the reactions stopped by rapid filtration through Whatmann GF/B filters. The filters were washed with 3 × 2 mL of Tris-HCl (50 mM, pH 7.4), and the

radioactivity retained on the filters was measured by scintillation spectroscopy in 2 mL of scintillation fluid. All experiments were performed in triplicate and repeated at least three times.

Saturation analyses were performed for each receptor using at least eight concentrations of each radioligand (concentrations ranging from 0.05 to 10 nM). Dissociation constants (K_d) were calculated using the EBDA/LIGAND program.[21,22]

Displacement curves were constructed for each compound at each receptor using seven concentrations of the displacing agents (one data point per log unit of concentration: 10^{-11}–10^{-5} M). Displacement curves were analyzed using EBDA/LIGAND to calculate pK_i values.

Radioligands. Radioligands were purchased from New England Nuclear. The specific activities of [^{3}H]5HT and [^{3}H]-DOB were 29.7 and 15.0 Ci/mmol.

Tissue Preparation and Incubation for Measurement of IP_3 Production. $5HT_{2C}$ receptor-mediated stimulation of IP_3 production was measured in the choroid plexus of the rat. The choroid plexus was removed, placed in 200 μL of oxygenated Krebs solution, and incubated with 0.85 nmol of myo-inositol and 0.35 nmol of [^{3}H]myoinositol for 1 h at 37 °C. During this incubation, the tubes were gassed with 95% oxygen/5% CO_2 every 20 min. A mixture of LiCl and pargyline was then added (final concentration: LiCl = 10 mM, pargyline = 10 μM) and 10 min later the test compounds (final incubation volume = 250 μL). Dose–response curves were constructed from data obtained from three separate measures per data point. The mixture was incubated for a further 0.5 h at 37 °C. The assays were stopped by the addition of 25 μL of a stopping solution ($HClO_4$ 2.64 N + EDTA 40 mM). Assay tubes were frozen on dry ice for 15 min, thawed, and then kept on ice for 1 h. The tubes were then centrifuged for 20 min at 24000*g*. Then, 250 μL of the supernatant was removed and placed in Eppendorf tubes together with 25 μL of 4 M KOH. The samples were mixed well and kept on ice for 15 min. These samples were then recentrifuged for 15 min at 14 000 rpm. We removed 230 μL of supernatant and added 30 μL of phytic acid. The isolation of IP_3 was as described in a previous report.[23]

A concentration response curve was constructed for 5HT, mCPP, and several synthesized compounds. Six concentrations were used per test compound with the highest concentration tested being 0.1 mM. The maximal effect produced by each compound was compared to the stimulation induced by 10 μM 5HT in order to calculate the relative intrinsic activity.

***In Vivo* Functional Test.** In the test used to evaluate $5HT_{2C}$ receptor agonism *in vivo*, elicitation of penile erection was determined in RORO rats (Biological Research Laboratories, CH-4414 Füllinsdorf, Switzerland). All drugs were dissolved or microsuspended in 0.3% v/v Tween-80 in physiological saline. All drug solutions were freshly prepared and injected subcutaneously (sc) in a volume of 5 mL/kg body weight or administered orally in a volume of 10 mL/kg body weight. Control animals were injected with an equivalent volume of vehicle. When drug solutions were prepared from a salt of the compound, the doses refer to the weight of the salt. Eight rats were tested per dose and were individually placed in Plexiglas cages (30 × 25 × 10 cm) to allow counting over a 45 min observation period. When a substance was active in inducing penile erections, half-maximal effective doses (ED_{50}) were calculated by probit analysis. In those instances in which not all rats exhibited penile erection, then the approximate doses producing penile erection in half of the rats was used.

Schedule-Induced Polydipsia Task in Rats. Excessive drinking was induced in adult female RORO rats (Biological Research Laboratories, CH-4414 Füllinsdorf, Switzerland) through the use of a fixed-time operant schedule (FT-1 min). The rats were drug experienced and were food deprived overnight prior to each test session. The test apparatus consisted of a sound-attenuated chamber surrounding a Plexiglas test box (30 × 25 × 30 cm) which was equipped with a stainless-steel grid floor and a mechanism to permit the automatic delivery of one 45-mg food pellet (Formula A/I; P. J. Noyes Company, Inc., Lancaster, NH) each min into a food cup located within the apparatus. The test session was 1 h. The experimental compounds were given in 0.3% (w/v) Tween-80 in distilled water in a volume of 2 mL/kg body weight. Treatment was administered 30 min prior to the start of testing. The same group of 10 rats was used to test vehicle and each of the selected doses of a test compound (doses chosen at half-logarithmic units in the dose range 1–30 mg/kg). Test days alternated with training days on which the session proceeded in the same manner as on test days, except no treatment was given and no data were recorded. A bottle containing tap water attached to the test apparatus was always available during test sessions with intake measured to the nearest 1 g. Evaluation was done to compare the effect of each dose to that obtained for the vehicle condition using a two-tailed Wilcoxon test with a p-value of ≤ 0.05 accepted as statistically significant. The lowest dose tested which yielded a statistically significant difference to vehicle treatment (MED, minimum effective dose) was determined.

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References

(1) Pälvimäki, E.-P.; Roth, B. L.; Majasuo, H.; Laakso, A.; Kuoppamäki, M.; Syvälahti, E.; Hietala, J. Interactions of selective serotonin reuptake inhibitors with the serotonin $5HT_{2C}$ receptor. *Psychopharmacology* **1996**, *126*, 234–240.
(2) Jenck, F.; Moreau, J.-L.; Mutel, V.; Martin, J. R.; Haefely, W. E. Evidence for a role of $5HT_{1C}$ receptors in the antiserotonergic properties of some antidepressant drugs. *Eur. J. Pharmacol.* **1993**, *231*, 223–229.
(3) Glennon, A. R.; Jacyno, J. M.; Young, R.; McKenney, J. D.; Nelson, D. Synthesis and evaluation of a novel series of N,N-dimethylisotryptamines. *J. Med. Chem.* **1984**, *27*, 41–45.
(4) Nichols, D. E.; Oberlender, R.; McKenna, D. J. Stereochemical aspects of hallucinogenesis. In *Biochemistry and Physiology of Substance Abuse, Volume III*; Watson, R. R., Ed.; CRC Press: Boca Raton, 1991; pp 1–39.
(5) Wood, A.; Smith, C.; Szewczak, M.; Dunn, R. W.; Cornfeldt, M.; Corbett, R. Selective serotonin re-uptake inhibitors decrease schedule-induced polydipsia in rats: a model for obsessive compulsive disorder. *Psychopharmacology* **1993**, *112*, 195–198.
(6) Kalir, A.; Szara, S. Synthesis and pharmacological activity of fluorinated tryptamine derivatives. *J. Med. Chem.* **1963**, *6*, 716–719.
(7) Murakami, Y.; Saito, Y. Japanese Patent 01, 31,760, 1989; *Chem. Abstr.* **1989**, *111*, 115035t.
(8) Wender, P. A.; White, A. W. Methodology for the facile and regiocontrolled synthesis of indoles. *Tetrahedron* **1983**, *39*, 3767–3776.
(9) Ishikura, T.; Gunji, T. Japanese Patent 01,311,056, 1989; *Chem. Abstr.* **1990**, *112*, 216418w.
(10) Weygand/Hilgetag. *Preparative Organic Reactions*, 4th ed.; Martini, A., Ed.; Wiley: New York, 1970; p 185.
(11) Berendsen, H. H. G.; Jenck, F.; Broekkamp, C. L. E. Involvement of $5HT_{1C}$ receptors in drug-induced penile erections in rats. *Psychopharmacology* **1990**, *101*, 57–61.
(12) Hoffman, A. β-Phenylisobutylmethyl ketone and its derivatives. The synthesis of α- and of β-Phenylvalerianic acid. *J. Am. Chem. Soc.* **1929**, *51*, 2542–2547.
(13) Smith, J. G.; Massicotte, M. P. Synthesis of 1,1-dimethylindane derivatives having synthetically useful substituents at the 5- and 6-position. *Org. Prep. Proced. Int.* **1978**, *10*, 123–131.
(14) Corse, J.; Rohrmann, E. Preparation of some substituted β-phenylisovaleric acids. *J. Am. Chem. Soc.* **1948**, *70*, 370–371.
(15) Loudon, J. D.; Razdan, R. K. Rearrangement of chromanones and dihydrocoumarines by aluminium chloride. *J. Chem. Soc.* **1954**, 4299.
(16) Barco, A.; Benetti, S.; Pollini, G. P. Concerning the synthesis of 4-methoxyindan-1-one. *Org. Prep. Proced. Int.* **1976**, *8*, 7–11.
(17) Olivier, M.; Marechal, E. Etude de monomeres halogenes et de leur polimerisation cationique. I. Synthese de divers fluoroindenes. *Bull. Soc. Chim. Fr.* **1973**, *11*, 3092–3095. Olivier, M.; Marechal, E. Etude de monomeres balogenes et de leur polimerisation cationique. II. Synthese de divers chloroindenes. *Bull. Soc. Chim. Fr.* **1973**, *11*, 8096–8099.
(18) Hannig, E.; Heyde, R.; Matschiner, H. Zur Kenntnis einiger 2-aminomethylierter kernsubstituierter Indanone-(1). *Pharmazie* **1973**, *28*, 217–220.

(19) Budhram, R. S.; Palaniswami, V. A.; Eisenbraun, E. J. Steric effects in the synthesis of 1,7-Dialkylindanes. *J. Org. Chem.* **1986**, *51*, 1402−1406.
(20) Sleight, A. J.; Stam N. J.; Mutel, V.; Vanderheyden, P. M. L. Radiolabelling of the human 5HT$_{2A}$ receptor with an agonist, a partial agonist and an antagonist: Effects on apparent agonist affinities. *Biochem. Pharmacol.* **1996**, *51*, 71−76.
(21) McPherson, G. A. 1985, Biosoft, Cambridge.
(22) Munson, P.; Rodbard, D. LIGAND: A versatile computerized approach for the characterization of ligand-binding system. *Anal. Biochem.* **1980**, *107*, 220−239.
(23) Bourson, A.; Wanner, D.; Wyler, R.; Petit, N.; Zwingelstein, C.; Rudler, A.; Sleight, A. J. Pharmacological evaluation of the discriminative stimulus of mCPP. *Pharmacol. Biochem. Behav.* **1995**, *53*, 107−114.

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